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CHANGES IN NUMBER OF CIRCULATING LEUKOCYTES IN RELATION TO SPONTANEOUS RECOVERY FROM PNEUMOCOCCIC INFECTION

EXPERIMENTAL ANIMAL USED: GUINEA PIG

MOYER S. FLEISHER, M.D.
AND
G. T. RICH, M.D.
ST. LOUIS

In a previous publication [1] the sequence of the cellular reactions occurring in the peritoneal cavities of guinea pigs that had been given intraperitoneal injections of pneumococci was reported. The relative proportions of the various types of cells were noted, and also the percentage of leukocytes showing phagocytosis. It appeared that no differences could be noted in these reactions in animals which died as contrasted with those which spontaneously recovered.

The study of celluiar reactions in relation to death or spontaneous recovery has been extended to the response of the blood leukocytes during the course of infection.

As in the earlier work, each guinea pig was given an intraperitoneal injection of 0.25 cc. of eighteen hour serum-dextrose-broth cultures of Pneumococcus type I (three tubes were used in order to compensate for possible variation of the organisms in individual tubes). Peritoneal exudate was examined (by cell counts and culture), and counts of the leukocytes in the peripheral blood were made at six hours, at twenty-four hours and at daily intervals through the fifth and sixth days and at suitable intervals thereafter. The specimens of peripheral blood were taken from the ear veins. Only the counts on peripheral blood are considered here.

Of the 92 guinea pigs considered in this series, 49 died and 43 survived; the latter lived at least thirty days after infection and took on weight. The mortality was almost the same in the male and the female animals. However, the weight of the animal at the time of infection was a factor in survival: of 23 animals weighing between 200 and 300 Gm., 18 died (about 78 per cent), while of 69 animals weighing over 300 Gm., only a few of which weighed more than

From the Department of Bacteriology, St. Louis University School of Medicine, and the Laboratory of the Jewish Hospital.

1. Robben, F. J.; Rich, G. T., and Fleisher, M. S.: Arch. Path. **20**:46, 1935.

400 Gm., 31 died (about 45 per cent); of the 23 animals weighing between 200 and 300 Gm., 17 died within the first forty-eight hours after injection of the pneumococci. Of the animals that died, 22 succumbed within twenty-four hours, 14 between twenty-four and forty-eight hours, 4 during the third day, 4 during the fourth and fifth days and 2 on the sixth to eighth days.

Among the surviving animals, 4 showed negative peritoneal cultures at twenty-four hours; 4 at forty-eight hours; 3 at seventy-two hours; 14 at four days, 7 at five days, 4 at six days, 3 at seven days, 3 at eight days and 1 at nine days.

Only 13 of the 49 animals which died lived beyond forty-eight hours. Certain of the animals which died after six days had negative peritoneal cultures before they died; this phenomenon had been noted in earlier experiments.

Among 43 animals which survived, a few (4) apparently possessed a relatively high degree of resistance to the organism, since the peritoneal cultures were negative at twenty-four hours. It may also be assumed that the 7 animals which showed negative cultures on either the second or third day had a fairly high degree of resistance. It is interesting to note the large number of animals which showed negative cultures on the fourth or fifth day, actually about 50 per cent of the surviving animals. By the eighth or ninth day all the surviving animals showed negative cultures.

In order to study the relations between the numbers of circulating white blood cells and survival or death of the guinea pigs, the experimental animals have been divided into six groups as follows: *A*, those animals which died in twenty-four hours (22 animals); *B*, those animals which died after twenty-four hours but before forty-eight hours (14 animals); *C*, those animals which died later than forty-eight hours (13 animals); *D*, those surviving animals whose peritoneal cultures became negative from the fifth through the ninth day (18 animals); *E*, those surviving animals whose peritoneal cultures became negative on the fourth day (14 animals); *F*, those surviving animals whose peritoneal cultures became negative before the fourth day (11 animals).

For the purpose of further consideration, the mean or average, white blood cell count of each of the six groups of animals is taken for comparison, and the counts in individual animals are not discussed, since the variation in the individual animals within the group is wide.

The mean of the total white blood cell counts obtained in 301 counts on 130 normal animals was $10{,}138 \pm 3{,}890$ per cubic millimeter. The limits of variation were between 2,000 and 28,000 per cubic millimeter. The mean of the neutrophil counts in 266 counts on 130 normal animals was $4{,}694 \pm 2{,}490$, and the limits of variation were between 300 and 20,000 per cubic millimeter. It is evident that there are wide variations in different animals, and it should be noted that there are also wide variations in individual animals in consecutive counts. In the cases of 159 normal animals the variations between consecutive counts were noted and the average of the variations of total white blood cell counts and neutrophil counts was $+517$ and $+356$, respectively ($+$ indicating an increase). The limits of variation between the consecutive counts in the same animals ranged between an increase of 15,000 and a decrease of 10,000 in the total white blood cell counts, and an increase of 13,000 and a decrease of 12,000 in the neutrophil counts.

TOTAL LEUKOCYTE COUNTS AND NEUTROPHIL COUNTS IN THE FIRST TWENTY-FOUR HOURS AFTER INFECTION

In table 1 are shown the mean, or average, counts in the six groups of animals (both total white blood cell counts and neutrophil counts) taken just before the infection, at six hours and at twenty-four hours, and also the mean, or average,

TABLE 1.—*Average Total Leukocyte Counts and Neutrophil Counts For Groups of Guinea Pigs Infected Intraperitoneally with Pneumococcus Type I*

	Total Leukocytes per Cubic Millimeter						Neutrophils per Cubic Millimeter					
	Average Count			Average Difference Between Counts			Average Count			Average Difference Between Counts		
	0 Hr.	6 Hr.	24 Hr.	0-6 Hr.	0-24 Hr.	6-24 Hr.	0 Hr.	6 Hr.	24 Hr.	0-6 Hr.	0-24 Hr.	6-24 Hr.
Group A (22 animals) Died within 24 hours.....	10,863	8,682	.....	—2,181	.....	.....	6,030	6,831	.....	+ 801	.....	.....
Group B (14 animals) Died within 24-48 hours.....	10,135	8,642	5,614	—1,493	—4,521	—3,038	4,392	6,052	2,854	+1,661	—1,538	—3,199
Group C (13 animals) Died after 2d day.....	9,984	8,284	6,246	—1,700	—3,738	—2,038	4,361	5,730	4,169	+1,369	— 192	—1,561
Group D (18 animals) Recovered after 4th day.....	9,863	9,200	11,294	— 666	+1,428	+2,094	4,238	5,833	7,111	+1,595	+2,873	+1,278
Group E (14 animals) Recovered on 4th day.....	11,714	11,571	15,477	— 133	+3,763	+3,906	5,200	7,821	8,885	+2,621	+3,685	+1,064
Group F (11 animals) Recovered before 4th day....	11,300	14,173	11,927	+2,873	+ 627	—2,246	4,354	9,364	6,045	+5,010	+1,691	—3,319

increases or decreases between counts at zero and six hours, zero and twenty-four hours, and six and twenty-four hours.

While there are of course some variation between the mean counts in the six different groups before injection, they are not of sufficient magnitude to be considered significant, so that it may be assumed that in these respects (total leukocyte counts and neutrophil counts) the groups as a whole were relatively homogeneous.

If one considers the mean total counts at six hours, it becomes evident that certain differences are suggested as between groups E and F, on one hand, and the other four groups, on the other hand. In the four latter groups of animals, with 3 dying eventually and 1 recovering only after the fourth day, the total counts tend to fall, while in the two groups in which the animals recover within the first four days after infection the total counts rise (F) or show very little change (E). It cannot be stated that these figures or the changes in the total counts summarized in these figures are statistically significant,[2] but there is the suggestion that even at this early period after the injection of the micro-organisms the tendency toward survival or the tendency toward death begins to be expressed in the change of the total leukocyte count. It is probably of some importance to note that in the three groups of animals which died and in the one group of animals which recovered only after the fourth day the mean total leukocyte counts are almost identical. In the column of table 1 showing the mean increases or decreases of the total white blood cell counts during the interval between zero and six hours, it will be noted that there is a gradation in the changes in the total leukocyte count from group A, in which the animals died within twenty-four hours and showed the most marked decrease, through group F, in which the animals showed negative peritoneal cultures before the fourth day and also a distinct increase in the total leukocyte count.

At twenty-four hours after infection only five groups are to be considered, the animals of group A having died before this count was made. It will be noted that at this time the mean total leukocyte counts are relatively low in the two groups which eventually died, but the mean total counts in the groups which survived are higher than at the beginning of the experiment. In addition, groups B and C both show a fall in total counts while the three other groups (which survived) show an increase in total counts. It can be stated that the changes in numbers of leukocytes at this period are significant practically regardless of what groups one compares. Thus one may compare group C with group E, in both of which the infection continued for at least thirty-six to forty-eight hours or more beyond the time at which counts were made, and one finds that the changes in these groups are significant. Or one may compare groups B and C with groups E and F (excluding those animals which showed negative cultures at twenty-four hours) and again the differences in the changes of total leukocyte counts are significant. Even when groups C and D are compared, a significant difference in the changes in the total counts can be observed, although in both groups infection continued subsequently for a considerable period of time before death or recovery intervened. It is evident that at twenty-four hours after the infection the total leukocyte count of the animals tends to show a positive correlation with eventual recovery or death, even though the outcome may be delayed until long after this particular time.

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2. The statistical significance of the changes (increase or decrease) of the total and the neutrophil counts has been studied by means of the Chi square formula, and whenever the term "statistically significant" is used it applies to this means of determination.

In group F it will be noted that the change in the total count at twenty-four hours is relatively slight when compared witih the original count. In this group apparently the maximum stimulating effect of the infection and the response of the invaded organism are past, and the total leukocyte count tends to return to normal.

In the case of the neutrophils it may be noted that at six hours in all the groups there is an increase in the number of these cells per cubic millimeter; the change in this count, therefore, does not in every group move in the same direction as does the change in the total count. There is a suggestion of a graded change in the neutrophil counts from the animals which died early through those which recovered and showed negative cultures early. When a comparison is made between groups B and C, on one hand, and groups E and F, on the other hand (excluding those animals which showed negative cultures at twenty-four hours), there is a significant difference in the changes of the neutrophil counts at six hours. No such difference can be noted in comparing other combinations of groups or in comparing group C with group D.

When the neutrophil counts at twenty-four hours are studied, it is immediately apparent that there is a marked difference between the two groups which will die (B and C) and the three groups which will survive (D, E and F). In the former two the numbers of neutrophils per cubic millimeter have diminished since the six hour count and even since the preliminary count, and this decrease is most marked in group B, which will die within the succeeding twenty-four hours. In the three groups which will survive the neutrophils are present in larger numbers than at the original count; in groups D and E there has occurred an increase since the six hour count, while in group F, in which the animals are on the way toward recovery, the neutrophil count has fallen since the sixth hour. It may be stated that significant differences can be noted between groups B and C as compared with groups E and F (omitting the animals that gave negative cultures at twenty-four hours) and also between the three surviving groups as compared with the two groups which died. It is questionable whether there exists a significant difference between groups C and D.

RATIO OF YOUNG TO OLD NEUTROPHILS

The ratio of young to older neutrophils has been studied in these animals before infection, at six hours after infection and at twenty-four hours after infection; the mean ratios are shown in table 2. All segmented neutrophils were classed as old and others as young cells.

TABLE 2.—*Ratio of Young Cells to Older Cells (Neutrophils) at Intervals After Infection of Pneumococcus Type I*

	0 Hr.	6 Hr.	24 Hr.
Group A—died within 24 hours	0.0549	0.321	.....
Group B—died within 48 hours	0.0609	0.206	1,352
Group C—died after 48 hours	0.100	0.323	0.967
Group D—recovered after 4th day	0.0503	0.412	0.402
Group E—recovered on 4th day	0.0654	0.210	0.167
Group F—recovered before 4th day	0.0624	0.176	0.166

It will be noted that the ratios existing before the injection of the bacteria bear no relationship to the possibility of survival or death of the animals. This is even more striking when the ratios in individual animals are observed.

At six hours after infection the ratio is increased in every animal with only a few exceptions (and these exceptions are found among both those which died and

those which survived). The mean ratios in the six groups are somewhat varied, but it is probable that the differences are not significant. In groups E and F, which recover earliest, the ratios tend to be lower but not strikingly so.

At twenty-four hours in the two groups which die (B and C) the mean ratios have continued to increase, while in groups D, E and F they have remained stationary or tend to fall.

It is evident that at six hours the ratio of the neutrophils bears little or no relationship to the eventual outcome of the infection but that at twenty-four hours the animals showing a lesser ratio of the young cells are likely to recover. Once again, however, the change in the ratios between the various time intervals seems to be more indicative than the actual figures.

TOTAL LEUKOCYTE COUNTS AND NEUTROPHIL COUNTS IN LATER STAGES OF INFECTION

In the later stages of infection, when the mean counts in the various groups are compared there is observable a tendency to maintain the differences in both total and neutrophil counts (tables 3 and 4) that have been observed during the first twenty-four hours; i.e., the counts of the surviving animals are higher than those of the animals which do not survive. In these later periods only groups C (died) and D and E (survived) are considered.

In group C, when one considers the mean or average of counts in all animals, it will be noted that the total cell count tends to remain below 10,000, rising to or above this number at about the fifth or sixth day. The longer the survival the greater the likelihood that the total leukocyte count will show an increase. There is a tendency for the count to rise on the day before death. A similar curve may be observed in connection with the neutrophil count in this group, the tendency to remain low and the tendency toward a rise in the later periods. In addition, lower counts are observed for the animals which die before the fourth day.

In group D (a rather heterogeneous group as regards the day of the first negative culture—ranging from the fifth to the ninth day) there is considerable irregularity in the average total count, but this is higher than in group C. The count after the sixth hour is constantly above 10,000 cells per cubic millimeter. In general, there is a rise of the total count on the day before the negative culture. The counts are usually lower in the lots which show the least rapid recovery (those showing negative cultures on the eighth or ninth day). Subsequent to the negative culture there is from day to day considerable fluctuation of the total count (shown both individually and collectively); this fluctuation subsequent to recovery is more marked in this group than in group E. The neutrophil counts in group D also are, on the average, higher than those in group C and tend to remain above 5,000 per cubic millimeter. In the smaller lots of group D the tendency toward association of higher counts with early recovery is evident, and low counts (below 5,000 per cubic millimeter) are noted particularly in the lot which required the longest period to achieve recovery.

Group E is a homogeneous group; in all animals the peritoneal cultures were negative on the fourth day. During the period of infection the average total count tends to be slightly higher than the count in either of the other two groups, and on the day of recovery (negative peritoneal culture) there is a tendency toward a fall of the total count. In the subsequent period there is less irregularity of the total count than in group D. These same three facts hold in relation to the neutrophil count in this group. In this group, in which relatively early recovery takes place, it appears that, with the cessation of the infection and when, therefore,

TABLE 3.—*Average Total Leukocyte Counts of Groups of Animals Dying After the Second Day and of Groups of Animals Recovering on or After the Fourth Day*

	0 Hr.	6 Hr.	1 Day	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days	8 Days	9 Days	10 Days	11 Days
Died after 2d, before 3d day....	14,300	10,400	5,900	9,100	.....	.....	.....	.....	.....	.....	.....	.....	.....
Died after 3d, before 4th day...	7,600	5,700	7,700	4,600	6,800	.....	.....	.....	.....	.....	.....	.....	.....
Died after 4th, before 5th day..	9,600	12,700	6,400	14,700	7,900	6,000	.....	.....	.....	.....	.....	.....	.....
Died after 5th day.....	10,900	8,900	5,000	7,700	6,500	9,800	9,400	12,700	.....	.....	.....	.....	.....
Group C.....	10,200	8,300	6,200	8,300	7,200	8,700	9,600	12,700	.....	.....	.....	.....	.....
Negative* on 8th or 9th day..	10,100	9,200	11,200	15,700	8,900	9,200	10,000	16,400	10,100	11,300	10,000	.....	9,300
Negative* on 7th day.....	9,800	7,700	17,400	11,600	16,900	14,200	14,100	.....	18,700	.....	12,100	24,400	17,000
Negative* on 6th day.....	10,700	7,500	10,400	14,300	10,500	15,200	18,400	17,200	16,400	20,200	11,300	.....	27,000
Negative* on 5th day.....	9,400	11,500	11,400	11,300	10,200	16,000	16,700	17,300	8,300	23,500	19,000	18,000	5,900
Group D.....	9,900	9,400	12,200	13,000	11,500	14,100	15,000	17,100	12,800	19,800	15,200	21,200	14,800
Group E (negative* 4th day)...	11,700	11,600	15,500	12,700	14,500	11,600	11,600	14,000	13,200	13,100	.....	16,200	10,800

* A culture of material from the peritoneal cavity was negative.

TABLE 4.—*Average Neutrophil Counts of Groups of Animals Dying After the Second Day and of Groups of Animals Recovering on or After the Fourth Day*

	0 Hr.	6 Hr.	1 Day	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days	8 Days	9 Days	10 Days	11 Days
Died after 2d, before 3d day.....	3,500	7,800	3,400	1,800	.....	.....	.....	.....	.....	.....	.....	.....	.....
Died after 3d, before 4th day.....	3,800	4,400	5,500	1,800	1,500	.....	.....	.....	.....	.....	.....	.....	.....
Died after 4th, before 5th day.....	4,300	8,100	4,800	7,700	3,700	4,100	.....	.....	.....	.....	.....	.....	.....
Died after 5th day.....	5,300	7,100	2,700	7,400	4,200	5,800	6,200	9,600	.....	.....	.....	.....	.....
Group C.....	4,300	6,000	4,000	4,600	2,900	5,100	6,200	9,600	.....	.....	.....	.....	.....
Negative* on 8th or 9th day.....	4,300	5,500	4,800	7,400	2,800	4,000	3,200	5,200	2,800	4,100	2,800	.....	6,200
Negative* on 7th day.....	3,600	1,900	11,300	4,000	3,000	9,700	6,900	.....	11,300	.....	5,100	13,400	6,300
Negative* on 6th day.....	4,500	4,900	6,800	8,800	4,700	6,600	11,500	7,500	5,900	11,500	4,000	.....	10,800
Negative* on 5th day.....	4,400	7,400	6,800	8,500	5,200	7,200	9,300	7,900	5,800	9,600	11,600	9,000	1,800
Group D.....	4,200	5,800	7,100	7,500	4,200	6,500	8,100	7,100	7,300	9,300	8,300	11,200	6,240
Group E (negative* on 4th day).....	5,200	7,800	8,900	6,800	6,000	4,700	4,800	5,100	4,900	4,900	.....	6,800	4,600

* A culture of material from the peritoneal cavity was negative.

there has not been too great injury to the macro-organism, the restoration of equilibrium as expressed in the total leukocyte and neutrophil counts takes place rapidly.

If one attempts to summarize the relation of the leukocytes to death or recovery, it may be observed that the mean counts of the total white blood cells of groups or of smaller lots and the counts of individual animals show a slight tendency to rise regularly on the day before recovery. Such rises, however, may be relatively insignificant or may have been preceded by rises at an earlier period. Particularly when individual animals are considered does this tendency toward a rise become less impressive. In group C, in which the animals did not survive, it may be noted that at the later periods (fourth and fifth days) there is also a tendency for the total and the neutrophil count to rise slightly, and this fact seems to lessen the significance of the rise in cells in the recovering animals.

In general, however, throughout the experiments higher total and neutrophil counts are observed for the animals which recover than for those which die. This is apparently a continuation of the leukocytic reaction which is evident at twenty-four hours after infection and is at least suggested even at six hours subsequent to infection.

COMMENT

The relationship of the changes in the total leukocyte counts and the neutrophil counts on the circulating blood to either spontaneous recovery or death subsequent to an experimental pneumococcic infection is undoubtedly complex. This relationship in the individual animal would not necessarily be a constant or direct one, since it must be assumed that the changes in the total leukocyte counts are not expressive of the sole factor concerned in the eventual outcome. Therefore it cannot be expected that in the case of any single animal the outcome of the infection can be prophesied on the basis of the changes occurring in these counts. These considerations justify the use of only the mean counts of various groups of animals in attempting to study these relationships.

An attempt has been made to interpret the results reported here by means of simple statistical treatment; only in a certain number of the comparisons has it been possible to demonstrate statistical significance of the differences noted. Certain of the material was such that statistical treatment was not feasible; a simple descriptive consideration of the results is therefore justified.

A study of the actual number of leukocytes or of neutrophils per cubic millimeter at various periods during the infection is not very satisfactory in giving information concerning the outcome of the infection. Particularly at six and twenty-four hours after infection the numbers of leukocytes or of neutrophils per cubic millimeter are not strikingly different in the six groups into which the animals have been divided. There is, however, even at six hours, both in total white blood cells and in neutrophils, a tendency toward higher numbers in the case of animals which recover earlier (within four days), and this becomes more marked at twenty-four hours.

In view of the fact that at the time of inoculation the mean total leukocyte and neutrophil counts are not the same in all groups, the change in the number of cells from time to time is more informative than the actual number at any one time. It can be observed that there is a graded sequence in the six groups of animals in the changes of both total leukocyte and neutrophil counts at six and at twenty-four hours. Certain irregularities in this gradation appear, but the difference even at six hours is demonstrable when comparing the two groups which died within seventy-two hours (A and B) with the two groups which recovered before or by the fourth day (E and F).

If one considers only those four groups in which death or recovery occurred later than twenty-four hours subsequent to the six hour count (B and C, which died, and D and E, which survived), the changes in the total number of leukocytes per cubic millimeter at this early period are more significant than those in the number of neutrophils. At twenty-four hours, however, changes in both the total number of cells and the number of neutrophils seem to bear a relationship to the outcome of the infection.

When the two groups which did not die or recover early (C, dying after seventy-two hours, and D, recovering after the fourth day) are compared, there is little difference between increases or decreases of cells at six hours; the change in the total count is more suggestive than that in the neutrophil count. At twenty-four hours, however, the changes are in line with the eventual outcome in relation to both total leukocytes and neutrophils. It may be suggested that in these two groups the factors relating to the eventual outcome which are evident in the changes in white blood cell counts are in the balance at six hours and become operative only at twenty-four hours.

In those animals which recover early (F) the total and neutrophil counts have risen markedly at six hours but have fallen at twenty-four hours. Apparently the reaction associated with the changes in leukocyte count has been brief and the leukocytic equilibrium tends to be restored fairly rapidly.

In following the counts in the experiments reported here, after the first day of infection there is evident a tendency for the total number of leukocytes per cubic millimeter to remain about 10,000 and for the number of neutrophils to remain above 5,000 in those animals which survive, while in the animals which die the numbers are usually lower. These numbers in surviving animals can hardly be considered as evidence of leukocytosis, since the mean for total leukocytes of normal guinea pigs is 10,138 and that for neutrophils 4,694. However, it is apparent that the leukocytic levels are better maintained in the recovering animals than in those which die.

In general, the total leukocytes tend to rise on the day before recovery or within a few days before recovery. While it is possible that this rise is associated with recovery, its importance is somewhat questionable, since the total leukocytes also rise in later stages of infection in animals which die after the fourth day.

The rise of the neutrophils on the day before recovery is less striking than that of the total leukocytes. In a number of the smaller lots the neutrophils do not rise at the time of recovery but rather tend to fall. In some of the smaller groups of animals dying at later dates the neutrophils tend to rise.

It is doubtful whether in later stages the changes in either total leukocytes or neutrophil counts can be definitely associated with recovery. As has been noted in connection with the relative numbers of cells in the peritoneal exudate in these infections,[1] it seems there is a cycle of cellular response which corresponds to the classic early neutrophil, later mononuclear reaction. It does appear that throughout the infection the leukocytic level is higher in the animals which will recover, but it is doubtful whether it is possible to associate specifically any change in the white blood cell picture with the point of recovery. It has been noted elsewhere [3] that the mononuclear cells tend to increase about the day before recovery, and this may be but a part of the general leukocytic increase, or it may be associated with the local mononuclear response (increase).

It has been recognized that in human beings a higher leukocyte level during the course of pneumococcic infection is associated with a more favorable prognosis;[4] as a rule the critical level in human beings has been assumed to lie above 15,000 or 20,000 per cubic millimeter. In regard to dogs Robertson and Fox [5] have shown that a large percentage of those with counts over 20,000 per cubic millimeter will survive, and in regard to rabbits Goodner [6] has shown that in connection with serum therapy the higher leukocyte count is associable with a better therapeutic effect. Our results in guinea pigs are in accord with these observations; namely, that the mean count for spontaneously recovering animals is higher than that for animals which succumb to the infection. However, it should be noted that in the guinea pigs the increase in the total leukocytes is distinctly less marked than in the human being and

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5. Robertson, O. H., and Fox, J. P.: J. Exper. Med. **69**:229, 1939.

6. Goodner, K.: J. Exper. Med. **60**:9 and 19, 1934.

the tendency is for the mean count of recovering animals only rarely to rise above 15,000 per cubic millimeter. In a few individual animals the total cells may rise to 30,000 per cubic millimeter and neutrophils to 27,000 per cubic millimeter. Goodner also has shown that in rabbits [6] the total number of leukocytes in the blood of the animals at the time of infection is an important factor in determining the outcome. It has not been possible to demonstrate a similar relationship between the total leukocytes or the neutrophils present at the time of initiating infection and death or survival in the guinea pig.

Probably the most striking relationship between leukocytes and the course of infection noted in our experiments with guinea pigs is the early change in the leukocyte count. In the studies referred to in the foregoing paragraph, emphasis is usually placed on the number of cells present at either the time of infection or at twenty-four hours after infection. In our experiments, at six hours after infection the trend in the leukocytic reaction associable with recovery or death becomes apparent or is suggested, most markedly in connection with the total leukocytes but also in connection with the neutrophils.

It is apparent that the reactions of the leukocytes which are generally associated with recovery from pneumococcic infection—i. e., higher counts—are initiated in the very early stage of the infection and become more distinct within the first twenty-four hours. The higher counts in the later stages are simply a continuation of these early reactions. These mechanisms of individual and nonspecific resistance of the animal which are associated with or expressed by the leukocytic reactions come into play in the very early periods of the infection.

It is not assumed that this change in the leukocytic picture is the determining factor in death or recovery. It may be an important factor (particularly the increased number of neutrophils) or it may be simply a manifestation of a more fundamental and more important physiologic activity of the invaded organism. The question is also open as to whether the increase or the decrease in the total leukocytes and neutrophils is to be considered as a varying degree of response to a stimulus, thus a varying degree of positive reactivity of the invaded organism, or whether it is to be considered as a picture resulting from greater or lesser degrees of intoxication and resultant depression of the host tissues, i. e., a passive, negative response.

A number of efforts have been made to study the constitutional or variable but preexistent reactions demonstrable in animals at or before the time of experimental infection in relation to resistance to infection. Essentially these studies may be considered as efforts to determine the nonspecific factors related to resistance. In recent years certain physiologic mechanisms, more readily demonstrable in the individual animal,

have been studied by Locke and his co-workers.[7] They demonstrated the fact that in rabbits the time required for a rise of 3 degrees in body temperature subsequent to chilling to 96 or 97 F. served to give a measure of the resistance of animals to a small dose of pneumococci. They associated this warming time recovery with circulatory responses and the adaptation of the animal in this regard.

It may be assumed that the early changes in the number of leukocytes which have been discussed in foregoing paragraphs might be an inherent quality of the physiologic mechanism of the animal, and with this in mind a number of animals were given intraperitoneally either aleuronat (an albuminoid substance and lecithin) or broth about four weeks before the experiment in which pneumococci were injected.

TABLE 5.—*Increase or Decrease of Total Leukocyte Counts and Neutrophil Counts at Six Hours After Injection of Broth and at Four and Seven Hours After Injection of Pneumococci*

	Total Leukocyte Counts			Neutrophil Counts		
Animal	6 Hr. Broth	4 Hr. Pneumococci	7 Hr. Pneumococci	6 Hr. Broth	4 Hr. Pneumococci	7 Hr. Pneumococci
137 C died within 24 hours	+1,800	+100	−2,800	+1,800	+2,800	+1,800
137 E died within 24-36 hours	+2,500	+200	+3,900	+4,700	+3,500	+7,300
137 A died within 36-48 hours	+1,300	+300	−1,900	+2,900	+ 900	+1,700
137 G died on 6th day	+ 200	−100	− 600	+ 900	+2,200	+3,900
Average for animals that died	+1,450	+125	− 450	+2,560	+2,350	+3,625
137 B recovered on 4th day	+4,600	+7,800	+8,700	+5,900	+7,200	+12,500
137 O recovered on 5th day	+5,200	+1,400	+9,300	+6,800	+1,300	+8,400
137 H recovered on 5th day	+1,000	+1,500	+2,900	+5,800	+4,100	+5,900
137 F recovered on 7th day	+1,500	+1,500	+2,400	+2,300	+3,000	+5,700
Average for animals that survived	+3,075	+3,050	+5,660	+5,150	+3,900	+8,125

The studies carried out with aleuronat as an irritant did not give results which could be satisfactorily correlated with the later results of the actual infection, since the distribution of surviving and dying animals in this series was such that conclusions could not be drawn. However, in all animals which subsequently died with pneumococcic infection the total cells had decreased at six hours after injection of aleuronat, the average change being — 2,900 cells per cubic millimeter, while in animals which survived the total cells either increased or decreased, and in those in which an increase was shown the average change was + 1,580 per cubic millimeter at six hours after injection of aleuronat. In all animals which survived the neutrophils were increased at six hours subsequent to injection of aleuronat, the average change being + 3,180 cells per cubic millimeter. One animal which died showed a decrease of 4,700 neutrophils per cubic millimeter.

In addition, in a single group of 8 animals a preliminary test was made by intraperitoneal injection of broth. Of the 8 animals, 4 died

7. Locke, A.: J. Infect. Dis. **60**:106, 1937; J. Immunol. **36**:159, 1939. Locke, A., and Main, E. R.: ibid. **36**:173, 1939. Locke, A.; Main, E. R., and Mellon, R. R.: ibid. **36**:183, 1939.

and 4 survived after the subsequent injection of pneumococci, four weeks later.

In table 5 is shown the distribution of the changes in the numbers of both total leukocytes and neutrophils at six hours after the injection of broth and at four and seven hours after the injection of pneumococci. It is evident that 100 per cent of the animals showing an increase of total cells greater than 3,000 per cubic millimeter at six hours after injection of broth survived, while 66 per cent of those showing a lesser increase died; 100 per cent of animals showing an increase of more than 5,000 neutrophils per cubic millimeter at this period survived, and 80 per cent of those showing less died. Since at twenty-four hours after the injection of broth the reaction had subsided, no differences can be noted as related to outcome of the infection at the time.

The increase or decrease of total leukocytes or neutrophils at four and seven hours subsequent to the injection of pneumococci also tends to show, particularly in group averages, the differences previously noted and is in general in line with the results discussed in foregoing paragraphs.

The results obtained in these experiments in which the reaction of the circulating leukocytes to the injection of broth was studied suggest that the early reaction of these cells is a part of the physiologic reactivity of the individual animal, so that this response of the macro-organism to stimulation by a noninfectious agent parallels in part, at least, the resistance of the animal to an infectious agent.

It is again evident from the experiments with injection of both broth and pneumococci and from those in which only the reaction to pneumococcic infection has been studied that the early leukocytic reaction alone will not serve to foretell the outcome of the infection; other as yet undetermined factors must have a part in the natural resistance, but certainly it seems that there is a positive correlation between early leukocytic reaction and death or recovery.

SUMMARY

The change (relative increase or decrease) of the total number of leukocytes and of the number of neutrophils per cubic millimeter of the circulating blood of the guinea pig twenty-four hours after infection with type I pneumococci bears a positive relationship to the spontaneous death or recovery of the animal. Even at six hours after infection this relationship is suggested or is evident. This positive relationship can be observed even when death or recovery is postponed for more than twenty-four hours subsequent to the six hour or twenty-four hour counts. Study of the response of animals to a nonspecific irritant suggests the possibility that this early reaction of the leukocytes is at least in part an inherent physiologic adaptation of the animal.

ATHEROSCLEROSIS

II. THE LIPIDS OF THE SERUM AND TISSUES IN EXPERIMENTAL ATHEROSCLEROSIS OF RABBITS

SIDNEY WEINHOUSE, PH.D.*
AND
EDWIN F. HIRSCH, M.D.
CHICAGO

The similarity between the lesions of human atherosclerosis and those produced experimentally in rabbits with diets containing cholesterol has been emphasized in many reports.[1] However, there are certain fundamental differences. Atherosclerosis in rabbits occurs with prolonged ingestion of cholesterol and only after significant cholesteremia has been present for some time. Cholesteremia has not been demonstrated convincingly as part of human atherosclerosis except in the specialized form associated with systemic xanthomatosis.[2] The deposition of the lipids in the arteries of rabbits appears to be part of a systemic lipid infiltration of the tissues,[3] comparable only with the conditions observed with general xanthomatosis in human beings. Although the high content of cholesterol in the blood is regarded as an important factor in the development of experimental atherosclerosis, the specific role of cholesterol is uncertain. Equally obscure is the significance of the quantitative distribution of the other lipids of the blood and of their relation to cholesterol.

The presence of appreciable amounts of lipids besides cholesterol in the lesions of human atherosclerosis [4] suggests that they, too, may participate in the evolution of the tissue changes. A significant relation

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From the Henry Baird Favill Laboratory of St. Luke's Hospital.

* The Seymour Coman Fellow of the Chemistry Department of the University of Chicago.

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1. (*a*) Anitschkow, N., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933. (*b*) Wacker, L., and Hueck, W.: Arch. f. exper. Path. u. Pharmakol. **74**:416, 1913. (*c*) Leary, T.: Arch. Path. **17**:453, 1934.

2. Müller, C.: Arch. Int. Med. **64**:675, 1939.

3. (*a*) Zinsserling, W.: Beitr. z. path. Anat. u. z. allg. Path. **71**:292, 1923. (*b*) Schönheimer, R.: Virchows Arch. f. path. Anat. **249**:1, 1924. Wacker and Hueck.[1b]

4. Meeker, D. R., and Jobling, J. W.: Arch. Path. **18**:252, 1934. Zeek, P. M.: Am. J. Path. **12**:115, 1936.

has been noted with human atherosclerosis in the approximate proportionality of the various lipid constituents in the lesions when compared with these elements in the circulating blood.[5] The close agreement between the lipid composition of the lesions and that of the blood suggests that the deposition in the blood vessels is nonspecific. Comparable studies in rabbits have not been made. The chemical analyses of atherosclerotic aortas of rabbits have been restricted to estimations of the cholesterol content.[6] Changes in the blood lipids of rabbits fed cholesterol likewise have received slight attention, the analyses, with few exceptions,[7] being limited to cholesterol.

Since hypercholesteremia in the rabbit is associated with an increase of the other lipid constituents of the blood,[7] the significance of these in the evolution of experimental atherosclerosis deserves investigation. The results reported here concern: (1) the variations of the blood lipids in rabbits fed cholesterol; (2) the changes in the lipid content of the aorta, liver, lungs, spleen, kidneys and adrenal glands in these animals; (3) an investigation of relations between the composition of the lipids of the blood and that of the lipids of the tissues, especially the aorta, and (4) a study of the morphologic changes of the tissues.

METHOD

Twelve rabbits, approximately 6 months old and weighing 2 to 2.5 Kg., were fed five times each week, Purina rabbit chow [8] containing 1 Gm. of solid cholesterol. A solution of cholesterol in ether was mixed thoroughly with a weighed quantity of the stock ration. The ether was evaporated slowly and the mixture stirred so as to distribute the cholesterol evenly. At the end of the day the regular food of the animals was replaced by a quantity of the prepared chow containing 1 Gm. of cholesterol. The rabbits received no other food until all the prepared chow had been eaten. A day or two of starvation was necessary before the rabbits would eat the cholesterol mixture. A control group of 7 rabbits was fed the stock Purina chow diet.

Serum Lipid Analyses.—At intervals of about twenty days 3 to 6 cc. of blood, depending on the lipid content, was collected from a marginal ear vein. After clotting and centrifugation, the serum was extracted with alcohol and ether according to the method of Bloor.[8a] The extract was filtered and washed, and a solution in purified petroleum benzine (petroleum ether) was prepared essentially as described by Kirk, Page and Van Slyke.[9] Aliquots of this solution

5. Weinhouse, S., and Hirsch, E. F.: Arch. Path. **29**:31, 1940.

6. (*a*) Eberhard, T. P.: Arch. Path. **21**:616, 1936. (*b*) Huber, M. J.; Broun, G. O., and Casey, A. E.: Proc. Soc. Exper. Biol. & Med. **37**:441, 1937. (*c*) Bauman, C. A., and Rusch, H. P.: ibid. **38**:647, 1938.

7. (*a*) Page, I. H., and Bernhard, W. G.: Arch. Path. **19**:530, 1935. (*b*) Wacker and Hueck.[1b]

8. The Purina chow is a commercial rabbit food consisting of various ground grains and minerals in pellet form.

8a. Bloor, W. R.: J. Biol. Chem. **77**:53, 1928.

9. Kirk, E.; Page, I. H., and Van Slyke, D. D.: J. Biol. Chem. **106**:203, 1934.

were used for the various analyses. The purified petroleum benzine solutions were slightly cloudy in spite of careful filtration. This precipitate, the nature of which is unknown, settled out completely on standing overnight.

Free and total cholesterol were determined by a slight modification of the method of Schönheimer and Sperry,[10] the color reading being made with a photoelectric colorimeter. Phospholipid was separated from another aliquot by Bloor's[11] method, and phosphorus was determined according to Fiske and Subarrow.[12] Total lipid was determined by oxidation with dichromate according to Bloor.[5a] The neutral fat per hundred cubic centimeters of serum was calculated by means of the following formula: $3.6\ N = T - (3.8\ C + 3.7\ CE + 3.0\ P)$ wherein N, T, C, CE and P are, respectively, the neutral fat, the milliliters of tenth-normal dichromate used, the cholesterol, the cholesterol esters and the phospholipids per hundred cubic centimeters of serum. The total lipid is the sum of all four fractions. The coefficients in the foregoing formula refer to the number of milliliters of tenth-normal dichromate required per milligram for the oxidation of the pure substances.

TABLE 1.—*Tissue Lipids of Control Rabbits* *

	Free Cholesterol		Total Cholesterol		Cholesterol Esters		Phospholipids		Neutral Fats		Total Lipid		Moisture		Percentage of Body Weight	
	Mean	S. D.†	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S.D.
Lung.....	0.48	0.04	0.52	0.06	0.06	0.06	3.42	0.36	1.38	0.41	5.33	0.39	80.7	0.6	0.49	0.0
Kidney....	0.31	0.05	0.34	0.05	0.04	0.05	2.90	0.52	2.45	0.71	5.90	0.87	80.5	1.8	0.59	0.07
Aorta.....	0.19	0.02	0.22	0.05	0.06	0.07	1.01	0.16	4.25	1.60	5.51	0.91	68.6	2.5	.....	...
Liver.....	0.19	0.02	0.29	0.06	0.16	0.10	3.44	0.29	1.85	0.36	5.65	0.30	74.4	1.8	3.71	0.2
Adrenal...	0.60	0.15	8.97	3.2	14.15	5.0	4.44	0.72	11.14	0.50	28.54	5.8	57.8	7.5	0.017	0.00
Spleen.....	0.36	0.05	0.37	0.02	0.06	0.05	2.20	0.50	1.86	0.61	4.14	0.61	79.4	2.0	0.06	0.0

* The values are average values expressed as percentage of moist tissue.

† S. D. means standard deviation and refers to the formula of Fisher: $S.\ D. = \Sigma \sqrt{\frac{x - \bar{x}}{n - 1}}$

Tissue Lipid Analyses.—The rabbits were put to death by means of air emboli after varying periods of feeding. The tissues were removed and weighed, and after samples were taken for microscopic examination, the extraction and analyses of the lipids were carried out as described previously.[5] The thin aortas of the control rabbits could not be separated completely from the adventitia, and the analyses of the normal tissues, therefore, were influenced by small amounts of adventitial fat. This contains only negligible quantities of cholesterol and phospholipid. Accordingly, only the neutral fat fraction was affected. In the atherosclerotic aorta, however, the wall was thick enough for a clean separation of the intima and media from the adventitia; thus, the extracts of these aortas contained no extraneous lipid. So that the results would be comparable, the portion of the aorta from the heart to the inferior mesenteric artery was used.

Normal Tissues.—The average values and their standard deviations for the lipids of the control tissues studied are recorded in table 1. The levels of free cholesterol were low in the aorta and liver and only slightly higher in the spleen, lungs and kidneys. The highest levels were in the adrenal glands. In all the tissues the percentages were constant. Cholesterol esters were virtually absent in

10. Schönheimer, R., and Sperry, W. M.: J. Biol. Chem. **106**:345, 1934.
11. Bloor, W. R.: J. Biol. Chem. **82**:273, 1929.
12. Fiske, C. H., and Subarrow, Y.: J. Biol. Chem. **66**:375, 1925.

all except the adrenal gland, where the amounts were large, amounting to as much as 14 per cent. The phospholipid content was lowest in the aorta (only 1 per cent) and highest in the adrenal gland (4.5 per cent). Neutral fat was maximal in the adrenal gland (11 per cent). The percentage was lower in the other tissues and in general was more variable than the percentage of any other of the lipids. The values of the total lipids were relatively constant, being between 4 and 6 per cent, except in the adrenal gland, with an average of 28.5 per cent lipids.

The values for the lipids in normal rabbit tissues agree in general with those recently reported for kidney, lung, liver, adrenal and spleen by Aylward and Stott;[13] and the values for cholesterol, with those reported for kidney and liver by Page and Menschick.[14]

There was also close agreement with recent analyses of normal rat tissues. Chanutin and Ludewig[15] reported the following percental values for normal rat liver and kidney, respectively: free cholesterol, 0.23, 0.42; total cholesterol, 0.28, 0.47; "lecithin," 3.36, 3.04; total lipid, 3.55, 3.36.

RESULTS

General Behavior of Rabbits.—All rabbits on the cholesterol diet seemed healthy and gained weight during the first part of the experiment. After about one

TABLE 2.—*Serum Lipids of Normal and Cholesterol-Fed Rabbits* *

Cholesterol Fed, Days Fed	Free Cholesterol		Total Cholesterol		Cholesterol Esters		Phospholipids		Neutral Fat		Total Lipids	
	Mean	S. D.†	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
Normal	17.9	5.5	50.4	12.2	65.4	16.4	117	21.4	141	26	335	55
40	349	113	1,162	311	1,340	341	556	149	685	301	3,250	650
80	452	112	1,652	758	2,010	980	706	222	718	260	3,660	1,340
120	317	71	1,100	253	1,425	382	498	75	341	76	2,620	423
160	280	84	773	260	829	296	401	90	490	196	2,010	550

* All values are average values and are given in milligrams per hundred cubic centimeters of serum.

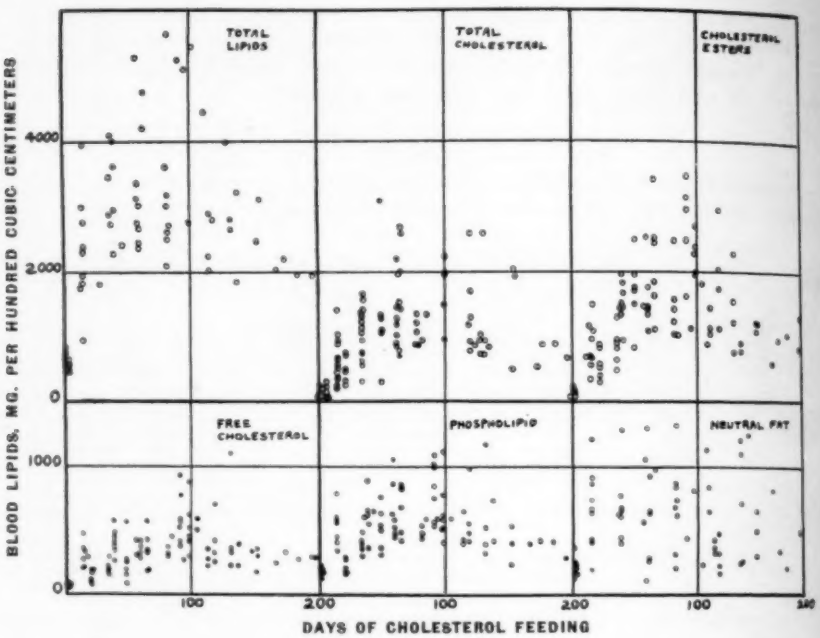
† S. D. means standard deviation and refers to the formula of Fisher: $S.D. = \Sigma \sqrt{\frac{x - \bar{x}}{n - 1}}$

hundred days the weight remained constant or decreased slightly. After about three months all rabbits had gray streaks in the cornea, which radiated from the pupil and increased with cholesterol feeding. In 8 of the 12 rabbits subcutaneous xanthomas of the paws developed, as described by Rusch, Baumann and Kline.[16] In rabbits 3, 4, 10 and 12 these lesions were large and attained a maximum diameter of about 4 cm. In rabbits 3 and 4 they had ulcerated within two months. There was no apparent relation between the severity of these lesions and the amount of cholesterol ingested, nor between the development of these xanthomas and the levels of the blood lipids. The levels of the blood lipids in 3 of the 4 rabbits in which these xanthomas did not develop were low (1, 2, 7) whereas one (5) had extremely high values for serum cholesterol and total lipid. Of the 4

13. Aylward, F. X., and Stott, W.: Biochem. J. **31**:2055, 1937.
14. Page, I. H., and Menschick, W.: J. Biol. Chem. **97**:359, 1932.
15. Chanutin, A., and Ludewig, S.: J. Biol. Chem. **102**:57, 1933.
16. Rusch, H. P.; Baumann, C. H., and Kline, B. E.: Arch. Path. **28**:163, 1939.

which had the most extensive lesions on the paws (3, 4, 10, 12), only one (12) had a markedly high lipid level. The fur, sleek and glossy at the beginning of the experiment, became rough and dry and could be pulled out in large quantities. After about three months the skin became scaly and certain regions with slight trauma, such as the back of the neck and areas on the hindquarters, became ulcerated.

Serum Lipids: Table 2 gives the normal serum lipid levels of rabbits and the variations with the cholesterol diet. The normal lipid levels of rabbits, especially those of the free and total cholesterol, were low. In spite of the differences in the methods of analysis, the values coincided closely with others reported.[17]



A diagram showing the distribution of the lipid constituents of the serum of the 12 rabbits on a diet containing cholesterol. Concentration of lipid is plotted against duration of cholesterol feeding.

Reports of the influence of fat in the diet on the absorption of cholesterol are confusing. Some believe cholesterol cannot traverse the intestinal wall without an added quantity of fat.[18] Our experiments and others[19] indicate that cholesterol absorption may occur on a low fat diet. Indeed, the high levels of blood lipids observed in our experiments and in others indicate that when the cholesterol is actually ingested, added fat has little effect on its absorption.

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17. Boyd, E. M.: Canad. J. Research (Sect. D) **16**:31, 1938. Page and Bernhard.[17a]

18. Duff, G. L.: Arch. Path. **22**:161, 1936; **20**:81, 1935. Schönheimer.[18b]

19. Turner, K. B., and Bidwell, E. H.: J. Exper. Med. **58**:115, 1933; **62**:721, 1935. Bruger, M., and Fitz, F.: Arch. Path. **25**:637, 1938. Meeker, D. R.; Kesten, H. D., and Jobling, J. W.: ibid. **20**:337, 1935. Huber, Broun and Casey.[19b]

There was a steady increase in all fractions of the serum lipids throughout the first forty or fifty days of the cholesterol diet. The highest levels were reached in fifty to one hundred days. The concentration then decreased somewhat or continued at a fairly constant level. The distributions of the lipid constituents of the serum during the course of feeding are plotted graphically in the accompanying chart. Although all the lipid fractions except the neutral fat had a similar pattern, there was considerable variation in the levels of the individual lipids. The sharpest rise occurred in the cholesterol ester, which at its peak was about thirty times the normal value. The increase in the phospholipids and free cholesterol, though considerable, was much less than that in the cholesterol esters. Thus, as the lipid concentration of the blood increased, there was a gradually increasing ratio between the cholesterol esters and the other lipids, this ratio reaching its highest value between the fiftieth and the hundredth day, when the concentrations of all the lipids were greatest.[20]

Coincident with the elevation of the blood lipids, there was marked turbidity of the serum. This condition increased with the rise of serum lipid concentrations, and at the highest lipid level the serums resembled milk.

Other Blood Changes.—Because anemia had been observed by Okey and Greaves[21] in guinea pigs on a cholesterol diet, the condition of the formed blood elements in the rabbits was investigated. There was no significant variation from normal in the hemoglobin or in the number of erythrocytes. As the proportion of cholesterol in the diet of the guinea pigs was much greater than that in the diet of the rabbits, a longer period of feeding or larger amounts of cholesterol may cause an anemia also in rabbits. An indication of this possibility and perhaps the reason for the anemia may be deduced from the xanthoma deposits in the bone marrow.[3b]

Tissues of Rabbits Fed Cholesterol.—(*a*) Aorta: With the exception of rabbit 1, fed cholesterol for only sixteen days, all rabbits on the cholesterol diet had atherosclerosis of the aorta of varying degree. An evaluation of the extent of the atherosclerosis is involved because of the variations in the extent of the deposits. In many animals the lining of the aorta had plaques fused into an almost continuous layer, which was thickest near the heart and diminished distally. In others the deposits in the aorta were nodular plaques, localized around the openings of branches. In 1 rabbit (12) the atherosclerosis was a single large lesion in the ascending portion of the aorta. The discussion will be confined, therefore, to a comparison of the chemical analyses. The lipid content is subject to the same criticism when used as an index of the severity of the atherosclerosis, but it has the virtue of being objective.

In all atherosclerotic aortas there was a marked increase in free and combined cholesterol, as well as a significant though variable increase in the phospholipids and neutral fat (table 3). The total lipids were increased from two to three times their value in the normal aorta. There was no direct correlation between the amount of cholesterol or other lipid in the aorta and the lipid level in the blood or the duration of feeding. Thus, rabbits 5 and 6 were fed about the same amount of cholesterol, and the serum cholesterol of rabbit 5 reached 2,200 mg. per hundred cubic centimeters, while that of rabbit 6 was 1,500 mg. per hundred cubic centimeters. The amounts of cholesterol deposited in the aortas were about the same.

20. The "neutral fat," consisting mainly of glycerids, was determined by difference. It therefore is the least accurately determined lipid, and the values of this fraction should be interpreted with reserve.

21. Okey, R., and Greaves, V. D.: J. Biol. Chem. **129**:111, 1939.

TABLE 3.—*Tissue Lipids of Cholesterol-Fed Rabbits**

	Rabbit	Duration of Feeding, Days	Sex	Free Cholesterol	Total Cholesterol	Cholesterol Esters	Phospholipids	Neutral Fat	Total Lipids	Moisture	Organ Weight, Percentage of Body Weight
Lung.....	1	16	F	0.46	0.46	0.00	3.25	1.00	4.70	81.8	0.43
	2	75	M	0.49	0.77	0.47	2.28	1.05	4.28	82.5	0.61
	3	83	F	0.58	0.76	0.30	3.18	0.97	5.04	80.1	0.39
	4	92	F	0.68	0.83	0.26	3.29	1.09	5.30	77.9	0.33
	5	97	M	0.60	1.25	1.10	3.69	1.38	6.77	79.1	0.32
	6	106	F	0.65	1.44	1.34	2.46	2.98	7.45	75.4	0.31
	7	125	F	0.63	1.00	0.62	3.34	1.49	6.08	79.8	0.35
	8	144	F	0.62	0.71	0.16	3.06	0.98	4.82	80.7	0.33
	9	155	M	0.65	0.79	0.24	4.13	1.34	6.37	80.5	0.36
	10	159	M	0.63	0.76	0.22	3.26	1.52	5.62	81.4	0.26
	11	186	F	0.56	0.84	0.47	3.09	1.03	5.15	80.2	0.32
	12	260	M	0.70	1.19	0.83	2.39	1.69	5.61	81.2	0.51
Kidney.....	1	16	F	0.30	0.32	0.03	2.57	3.15	6.04	80.5	0.74
	2	75	M	0.49	0.71	0.37	3.36	2.70	6.92	75.9	0.63
	3	83	F	0.44	0.61	0.29	2.85	2.15	5.73	83.3	0.64
	4	92	F	0.45	0.60	0.26	3.17	2.66	6.55	79.7	0.58
	5	97	M	0.48	0.83	0.59	3.29	2.03	6.39	80.1	0.54
	6	106	F	0.82	1.34	0.88	4.07	1.43	7.20	79.7	0.58
	7	125	F	0.48	0.81	0.56	3.71	0.80	5.55	82.4	0.48
	8	144	F	0.40	0.57	0.30	2.94	2.11	5.74	81.5	0.60
	9	155	M	....	....	....	....	....	....	....	....
	10	159	M	0.32	0.38	0.10	2.37	3.08	5.87	81.2	0.62
	11	186	F	0.41	0.63	0.37	3.18	5.05	9.04	75.8	0.48
	12	260	M	0.50	0.76	0.44	2.51	1.38	4.83	79.2	0.68
Aorta.....	1	16	F	0.19	0.19	0.00	1.22	3.11	4.53	70.1	....
	2	75	M	0.47	0.99	0.87	1.16	8.58	11.1	68.4	....
	3	83	F	0.92	3.18	3.81	1.39	4.63	10.8	67.8	....
	4	92	F	0.70	1.36	1.12	1.90	2.10	5.82	65.2	....
	5	97	M	1.84	5.86	6.79	2.91	3.92	15.5	63.2	....
	6	106	F	2.76	6.15	5.72	2.76	1.77	13.0	63.4	....
	7	125	F	1.06	2.56	2.52	1.81	7.56	13.0	65.7	....
	8	144	F	1.77	2.69	1.55	2.19	10.1	15.6	69.1	....
	9	155	M	2.11	5.00	4.87	2.52	8.09	17.6	64.0	....
	10	159	M	0.93	2.21	2.16	1.50	6.96	11.5	64.5	....
	11	186	F	2.64	3.45	1.36	2.72	12.00	18.7	69.5	....
	12	260	M	3.22	4.27	1.76	3.26	3.26	11.5	67.7	....
Liver.....	1	16	F	0.21	0.34	0.22	2.98	1.51	4.93	74.2	4.52
	2	75	M	0.57	1.27	1.18	3.96	1.27	6.98	78.1	3.52
	3	83	F	0.39	1.53	1.92	3.74	2.48	8.53	71.2	4.73
	4	92	F	0.46	1.59	1.90	4.06	1.86	8.28	75.2	3.96
	5	97	M	0.91	2.99	3.50	4.87	3.16	12.44	70.9	3.11
	6	106	F	0.63	2.59	3.30	4.97	1.97	10.9	71.8	3.41
	7	125	F	0.65	2.49	3.10	4.09	1.61	9.45	70.0	5.05
	8	144	F	0.46	1.31	1.43	4.36	1.98	8.23	72.4	4.16
	9	155	M	0.89	4.41	5.95	5.00	1.48	13.3	70.8	4.78
	10	159	M	0.51	2.17	2.80	3.38	2.72	9.41	72.6	4.46
	11	186	F	0.66	1.89	2.07	4.12	2.15	9.01	72.2	3.65
	12	260	M	0.97	3.64	4.51	2.07	3.97	11.5	74.7	3.15
Adrenal....	1	16	F	0.49	14.1	23.0	4.85	10.3	38.6	46.7	0.014
	2	75	M	1.18	19.9	31.6	3.27	9.65	45.8	42.1	0.027
	3	83	F	1.85	26.6	41.9	2.56	1.64	48.0	43.0	0.032
	4	92	F	1.08	16.4	25.8	3.46	19.4	49.7	41.9	0.038
	5	97	M	1.38	23.8	37.7	3.07	13.7	55.8	37.7	0.030
	6	106	F	1.47	22.0	34.6	3.22	3.34	42.6	47.0	0.015
	7	125	F	1.47	21.2	33.2	2.68	8.20	45.6	48.0	0.035
	8	144	F	1.12	19.3	30.7	2.00	10.2	44.0	45.3	0.025
	9	155	M	2.20	25.5	39.4	3.10	4.73	49.5	43.8	0.029
	10	159	M	2.09	15.7	22.9	2.31	3.79	31.2	....	0.025
	11	186	F	1.14	21.2	33.6	2.95	4.17	42.1	46.5	0.015
	12	260	M	1.44	15.0	23.0	2.12	0.00	26.5	43.5	0.027
Spleen.....	1	16	F	0.36	0.39	0.05	2.70	0.89	4.00	77.6	0.12
	2	75	M	0.69	0.78	0.15	2.29	1.82	4.95	81.9	0.08
	3	83	F	0.58	1.05	0.86	2.47	1.32	5.17	76.3	0.08
	4	92	F	0.46	0.74	0.48	2.67	1.02	4.63	78.5	0.06
	5	97	M	1.00	1.88	1.48	3.33	2.75	8.56	76.5	0.07
	6	106	F	0.11	1.43	0.88	2.64	1.61	6.04	77.5	0.09
	7	125	F	0.96	1.31	0.59	2.56	1.98	6.09	78.1	0.12
	8	144	F	0.47	0.63	0.26	2.34	1.12	4.19	79.2	0.10
	9	155	M	0.83	1.36	0.89	3.12	1.69	6.55	79.2	0.06
	10	159	M	0.55	1.29	1.25	2.99	0.12	4.90	81.1	0.03
	11	186	F	0.65	1.25	1.01	2.51	0.83	5.00	79.2	0.15
	12	260	M	0.89	2.11	2.06	2.13	1.57	6.63	77.0	0.04

* All values are given as percentages of moist tissue.

Again, rabbits 2 and 3 received equal amounts of cholesterol, and the serum lipids rose to the same level, 1,300 mg. per hundred cubic centimeters; but the amount in the aorta of the latter was thrice that of the former. There is no esssential difference in moisture content between normal and atherosclerotic aortas.

(*b*) Liver: The livers of all rabbits fed cholesterol, except rabbit 1, contained lipids substantially above those found in normal rabbits, the increase being greatest in the cholesterol ester fraction and least in the neutral fat fraction. As in the aorta, the lipid levels in the liver had no direct relation to those in the blood or to the amounts of cholesterol ingested. No definite correlation was noted between the lipid content and the gross appearance of the organ. With the exception of rabbits 11 and 12, which were fed the longest time, and rabbit 1, fed the shortest time, the livers of all the animals had a similar gross appearance. The red-brown lobules were distinct, and the margins were gray-yellow; thus there was produced a mottled brown and yellow color. The liver of rabbit 11 was dark red and was without the peripheral lobular markings. The capsular surface was rough, and the tissues were elastic. The liver of rabbit 12 was small and yellow-brown. In spite of the marked changes in the liver of rabbit 11, the lipid values were similar to those of other animals. The liver of rabbit 12 was high in cholesterol and neutral fat, but contained less phospholipid than any rabbit thus far studied. The moisture content of the livers was not significantly different from the normal, though the weight of most of the organs in relation to the body weight was somewhat greater than normal.

(*c*) Kidneys: The changes in the free cholesterol of all rabbits except rabbit 6 were minimal, and although the changes in the cholesterol ester fraction were larger, the increases were not nearly so marked as in the aortas and livers. No significant increase was observed in the phospholipid and neutral fat; the small increases in the total lipid over the normal values were due to the increased cholesterol. There were no changes in the moisture content or in the size of the kidneys. Gross changes were observed only in the kidneys of rabbits 6, 7 and 12. On the surfaces made by frontal hemisection, fine wedge-shaped deposits extended from the pelvis into the medulla and cortex, widening near the periphery. The greatest changes were in rabbit 6, in which these deposits occupied most of the surfaces. The capsular surface of the kidney also was deeply pitted. The kidneys of this rabbit contained the greatest amounts of free and combined cholesterol and phospholipid. The other kidneys with changes differed only slightly in lipid content from the grossly normal kidneys.

(*d*) Lungs: The lungs of all rabbits had no changes grossly. The moisture content was fairly constant and normal, though the weight varied widely. The only differences in lipid content were in the free and combined cholesterol, the latter accounting for most of the increase in the cholesterol fraction.

(*e*) Spleen: There were large variations in the size of the spleen. In some instances the tissues were dark red; in others, red or brown tinged with yellow. The free and combined cholesterol fractions were higher than normal, but the other lipids were not changed appreciably.

(*f*) Adrenal Gland: The adrenal gland was the only tissue which had a significant change in moisture content with cholesterol feeding. This value decreased from an average of 58 per cent in the normal to about 45 per cent in the cholesterol-fed rabbits. There was also a marked increase in size. Excepting rabbits 6 and 11, the weights in proportion to the body weight were about double the value for the normal rabbits. The free cholesterol increased from two to three times, and

the cholesterol esters, which even in the normal glands constituted 14 per cent, increased to twice the average, with the administration of cholesterol. The phospholipids were decreased slightly. The neutral fat, fairly constant at about 11 per cent in normal rabbits, varied in the cholesterol-fed animal from 0 to 17 per cent, and generally was considerably lower than normal. These results bear out those reported by Kay and Whitehead [22] for free and combined cholesterol, although the differences between the male and female animals reported by these workers were not observed.

The largest changes in lipid composition of the tissues with cholesterol feeding were in the liver, aorta and adrenal glands, whereas variations in the lungs, kidneys and spleen were minimal. The greatest changes occurred in the cholesterol ester fraction, though the free cholesterol was always increased. The phospholipids were increased in the aorta and liver, decreased in the adrenal gland and unchanged in the other tissues. The changes in the total lipid content were greatest in the liver, aorta and adrenal gland and were only slight in the other tissues. Only the moisture content of the adrenal glands was changed significantly by the cholesterol diet. This tissue alone increased appreciably in size with the cholesterol diet. There appeared to be no sex difference in the amounts of lipid deposited or in any other property of the tissues studied.

Relation of Lipid Deposition in Aorta to That in Other Tissues.—Table 3 shows with some exceptions that when the cholesterol content of the aorta is high, it is high also in the other tissues. This relation is noted especially in comparisons between the aorta and the liver, but not so well in comparisons between the aorta and the adrenal tissues. The same relations hold when the phospholipid contents of the aorta and liver are compared, but there is no relation between the phospholipid content of the aorta and that of the adrenal gland.

The results of the histologic studies are summarized briefly. The changes in the tissues agree with those published.[3b]

(*a*) Aorta: The intimal plaques of the aorta had a loose mesh of fibrillar tissue and aggregates of large granular phagocytes (lipophages). The lipid material was in coarse and fine granules in the sections of formaldehyde-fixed tissues stained with scarlet red. The subintimal portions of the media also had granular lipid deposits. The extent and thickness of the plaques corresponded with the gross appearance. In the large lesions some of the swollen phagocytes were necrotic, and the granular lipid material was extruded into the stromal portions of the plaque.

(*b*) Liver: From the earliest to the advanced stages of cholesterol feeding, the Kupffer cells of the liver contained quantities of granular lipid material. Increasing numbers of large mononuclear phagocytes laden with granular lipids appeared in the stroma of the portal canals. Gradually the liver cells became infiltrated. The stroma in the livers of some rabbits was increased, spreading from the portal and capsular tissues into the lobules.

(*c*) Kidney: Corresponding to the gray-yellow tissue noted grossly were interstitial deposits of granular lipid material, mainly in swollen mononuclear phagocytes. The regional tubules had disappeared. A few phagocytes in the glomerular tufts contained granular lipid material.

(*d*) Lungs: Large branches of the pulmonary artery had intimal plaques with lipophages, similar to the lesions of the aorta. There also were clusters of mononuclear lipophages in the alveolar walls associated with a little fibroblastic stroma. Along the pleura were other large lipophages.

22. Kay, W. W., and Whitehead, R.: J. Path. & Bact. **41**:293, 1935.

(*e*) Spleen: The pulp contained lipophages in proportion to the amounts of lipid deposited. With marked lipid deposits there were infiltrations of the trabeculae and walls of the blood vessels.

(*f*) Adrenal Gland: The cells of the adrenal gland were markedly enlarged and filled with lipid material. The cells in these tissues were directly infiltrated by the lipids.

(*g*) Other Structures: Fixed tissue phagocytes laden with lipids were observed in other viscera. The submucosa and stroma of the villi of the small bowel and the submucosa of the pyloric portion of the stomach contained dense masses of lipophages. The replacement of marrow tissues in the ribs by masses of large lipophages and the growth of xanthoma lesions on the feet were further manifestations. The main branches of the coronary arteries in the myocardium had intimal plaques of lipophages that reduced markedly the channels of these vessels.

COMMENT

The free and combined cholesterol of the blood of a rabbit in a typical experiment with a cholesterol diet increase during the first three months. These lipids later gradually decrease though the ingestion of cholesterol is continued. The level of cholesterol in the blood represents a balance between absorption, on the one hand, and storage, excretion and destruction, on the other. Analyses demonstrate that considerable amounts of cholesterol are deposited in the tissues. Metabolism, according to Page and Menschick,[14] accounts for about one third of the cholesterol ingested by the rabbit, but there is no information regarding the amount excreted. An increase in the rate of deposition, excretion or metabolism being unlikely, the subsequent decline in cholesterol content of the blood probably is due to an impairment of the absorption of cholesterol from the intestinal tract as a result of the marked deposition of lipids in the lining and wall of the stomach and bowel.

Others [23] have observed the coincident increase of the phospholipids and glycerides of the blood with cholesterol diets in rabbits. In those experiments the absorption of cholesterol from the bowel was facilitated by a large amount of oil. The diet of the rabbits in our experiments contained only the small amount (2.5 per cent) of fat present in a commercial rabbit food. The increase of the lipids in the blood, therefore, was due to the cholesterol diet alone.

The cause of the marked lipemia in rabbits with cholesterol diets has been sought in a destruction or binding of the lipase in the blood. Remezow [24] demonstrated an inhibition of serum lipase with cholesteremia, due to a specific binding of the active enzyme groups and the cholesterol. Binding of lipase seems to be a general property of alcohols.[25] Schönheimer's [3b] experiments, however, indicate that a

23. Wacker and Hueck.[1b] Schönheimer.[3b] Page and Bernhard.[7a]

24. Remezow, I.: Arch. f. d. ges. Physiol. **221**:534, 1929.

25. Murry, D. R. P., and King, C. G.: Biochem. J. **24**:190, 1930. Glick, D., and King, C. G.: J. Biol. Chem. **94**:497, 1931.

decrease in lipolytic activity of the blood alone does not explain the lipemia. In contrast with the soluble constituents of the blood, the lipids are dispersed as colloidal complexes. The speed and extent of enzyme reactions are affected directly by conditions that change the colloidal properties of the lipids. In the gradual development of experimental atherosclerosis in rabbits there is an increase of all of the blood lipids, associated with a disproportionate rise of the cholesterol ester fraction. An increase of a hydrophobic component, such as cholesterol or its esters, would decrease the dispersion of the lipid complex and would cause a marked reduction of the surface area exposed to enzyme activity. Thus, although the activity of the lipolytic enzymes remained unchanged, the decreased surface of the substrate would explain the accumulation of phospholipids and glycerides in the blood. The studies of Perlman and Chaikoff [26] on the effect of cholesterol in the formation and destruction of phospholipid in the liver of the rat support this view indirectly. With radioactive phosphorus as indicator, they noted that cholesterol caused a marked decrease of these changes. A disproportionately large amount of cholesterol in the lipid complex apparently is able to reduce the rate of phospholipid activity.

In discussions of the evolution of atherosclerosis the significance of the physical state of the plasma lipids has received scant attention. These lipids in the liquid portion of the blood are carried into the tissue spaces of the lining of vessels. An increase in the relative proportions of cholesterol or other hydrophobic substance would cause instability of the colloid dispersion and agglomeration into larger particles. This dynamic process occurs continuously in the plasma and therefore in the tissue spaces of the vessels. Tissue factors [27] not clearly understood at this time are important in determining the sites of lipid deposition.

As a consequence of the lipid precipitation, reactions are initiated in the tissues, such as phagocytosis, growths of fibrous tissues in response to the chemical action of the precipitated lipids, and alterations in nutrition which lead to the formation of atherosclerotic plaques.

SUMMARY

The lipids in the serum of rabbits maintained on a diet of commercial rabbit food containing approximately 1 per cent added cholesterol attain their maximum concentration in about three months. Thereafter they decline gradually although the cholesterol diet is continued.

When the serum lipids are at their maximum levels, the free cholesterol is increased twenty-five, the cholesterol esters thirty, the

26. Perlman, I., and Chaikoff, I. L.: J. Biol. Chem. **128**:735, 1939.
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phospholipid seven, the neutral fat five and the total lipid eleven times above the normal value.

The amount of total lipids in the aorta, liver, adrenal glands, lungs, kidneys and spleen is increased in these rabbits, especially the content in the first three tissues. The largest increase among the lipid fractions is in the cholesterol ester.

There is no direct relation between the degree and duration of the lipemia and the amount of lipid deposited in the tissues, although the composition of the lipids in the blood and tissues is similar.

Large amounts of lipids are contained in the fixed tissue phagocytes (lipophages) in the liver, spleen, lungs, aorta and large arteries, kidneys, bone marrow and subcutaneous tissues (xanthoma nodules). Lipids are deposited also into the hepatic and adrenal gland cells.

The physical state of the blood lipids is regarded as an important factor in the evolution of the experimental atherosclerosis induced in rabbits by diets containing cholesterol.

PHAGOCYTOSIS OF COLLAGEN

GEORGE A. VASSOS JR., M.D.
NEW YORK

The observation of cells apparently phagocytosing collagen in the bases of two large chronic prepyloric gastric ulcers raises several interesting questions. Is collagen actively phagocytosed during the healing of gastric ulcers, and, if so, why is this not seen more commonly in other reparative processes? It is well known that phagocytes ingest foreign bodies and necrotic debris, but a review of 100 cases of gastric ulcers and several hundreds of surgical pathologic specimens in this laboratory reveals no other instances of the phagocytosis of collagen.

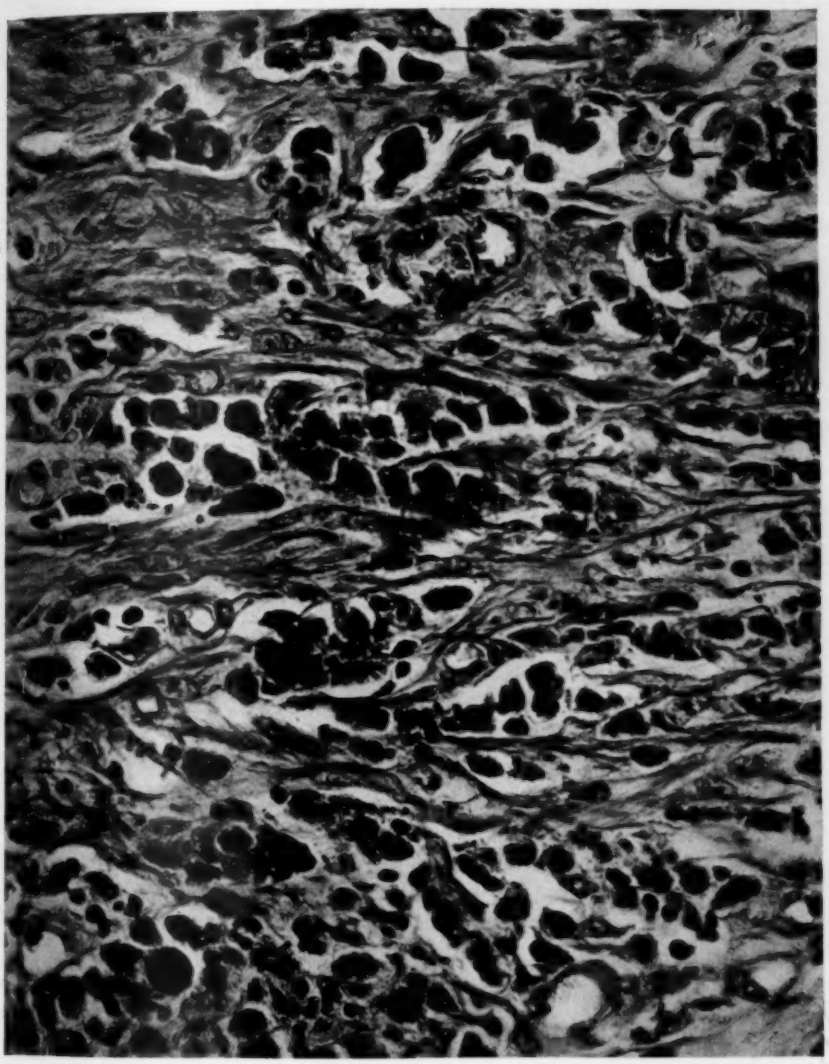
The material for this study consists of tissue taken through the edges and bases of two large prepyloric gastric ulcers removed by resection from men 44 and 58 years of age. Neither patient had any evident associated diseases. The first ulcer was 2 cm., and the second 2.5 cm., in diameter. Both were covered and partly filled in with adherent viscous bloody exudate, and were surrounded by the usual coxcomb frill of redundant tissue. On section the entire bed of each ulcer was composed of dense, yellowish white scar tissue. Blocks taken for routine microscopic study were fixed in a mixture of alcohol and solution of formaldehyde U.S.P.

Histologic study of the two ulcers revealed that the surfaces were covered with necrotic debris, numerous erythrocytes in a fibrinous coagulum, a moderate number of lymphocytes, a few neutrophilic polymorphonuclear leukocytes, a scanty number of eosinophils and plasma cells showing both intracellular and extracellular Russell bodies. The bases of the ulcers were composed of dense, moderately vascular, variably necrotic fibrous tissue, throughout which there were scattered a few remaining smooth muscle cells. In none of the sections was there any evidence of neoplastic change. The most striking feature in each lesion was the rather large, coarsely granular mononuclear cells interspersed throughout the dense fibrous stroma near the surface of the ulcer (figure).

These cells varied in diameter from about 8 to 30 microns but averaged about 20 microns and in form from somewhat round to roughly elliptic. There was a definite cell membrane around each cell. The

From the Department of Surgical Pathology, New York Hospital, and Cornell University Medical School.

nuclei tended to be central, were irregularly oval, measured about 7.5 microns or slightly more in their longer axis, and had a dense nuclear membrane in contrast with the less dense nuclear substance. The nuclei were simple, and the cells were always seen to be mononucleated. Their



Section through the base of a large chronic prepyloric gastric ulcer. The large cells appearing as phagocytes throughout the collagenous stroma contain coarse irregular granules (black in the photomicrograph), which have the same staining affinities as does collagen. In this section, which was treated with Van Gieson's stain, they are bright red. × 350.

most striking feature was their cytoplasm, which was characterized by definitely coarse irregular fragments, which appeared to be composed of granules of collagen. The size, shape and number of these were variable (figure). The staining qualities of the cytoplasm, as well as the morphologic appearance of these cells, led one to believe that they might be phagocytosing collagen from the dense stroma of the bases of the ulcers.

With Masson's stain [1] as modified by Goldner the dark green–staining coarse granules of these cells were seen to have an affinity for the green dye, as does collagen. In marked contrast were the orange-staining erythrocytes, the vermilion Russell bodies, the bright red granules of the Paneth cells and the light red granules of the eosinophils seen in the sections. Thus the green–staining cells were distinct from the aforementioned cells. Other staining technics were used in an attempt to identify the nature of the granules suspected of being ingested bits of collagen.

Stains for mucin [2] revealed none in the apparent macrophages, while the red–staining mucin in the goblet cells of the mucosa stood out in definite contrast. The cytoplasmic granules of the former appeared light heliotrope, as did the collagenous stroma.

With Van Gieson's stain [3] the granules of the probable macrophages were brilliant red and somewhat darker than the red–staining collagenous stroma (figure).

When Mallory's aniline blue stain [4] for collagen was used, these cells stood out as large, bright blue granular cells, which had an undoubted affinity for the aniline blue, as did the collagenous stroma of the base of the ulcer. This was similarly demonstrated with the use of the Masson trichrome stain [1] as modified by Goldner, which replaced the light green with aniline blue.

In sections subjected to a modified Giemsa technic [5] the bright red eosinophils and dark blue granular mast cells were quite distinct from the larger, light blue granular macrophages.

The ordinary hematoxylin and eosin stain demonstrated the granules of these cells to be definite, coarse and irregular, taking on a violaceous red slightly darker than that of the collagenous stroma.

Mallory's phosphomolybdic acid–hematoxylin stain [6] for collagen revealed the macrophages to be deep bluish purple granular cells, while the collagen was of a similar color.

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2. Mayer, P.: Mitth. a. d. zool. Station z. Neapel **12**:303, 1895-1897.
3. Van Gieson, I.: New York M. J. **50**:57, 1889.
4. Mallory, F. B.: Stain Technol. **11**:101, 1936.
5. Wolbach, S. B.: J. M. Research **41**:1, 1919.
6. Mallory, F. B.: Anat. Anz. **6**:375, 1891.

Identical affinity for the brownish red stain of the collagen was seen in the cytoplasmic granules of the apparent phagocytes with Mallory's phosphotungstic acid–hematoxylin technic.[7]

With the silver impregnation method of Foot [8] these cells became grayish red, while the collagen was impregnated red. It is interesting to note in passing that throughout the bases of the ulcers the network of argyrophilic reticular fibers was quite intact, being neither phagocytosed nor digested nor disintegrated by the reparative processes. It was as though the reticular network formed the basic structure for the framework of the healing processes.

It should be mentioned that the granular phagocytes appeared somewhat similar to cells seen to be the seat of hyaline degeneration in a cylindroma that was observed, a true basal cell skin tumor. Although the cells noted in the latter had some affinity for aniline blue, light green and acid fuchsin, they were hyaline rather than granular and took the dyes lightly in contrast to the granular, deeply staining phagocytes of the gastric ulcer.

Thus it has been shown that these macrophages, thought to be phagocytosing collagen, contained included granules that had the same affinity for the various dyes as does collagen. Therefore, it seems probable that the process seen was one of phagocytosis of the dense collagenous stroma in the base of the ulcer. One can only speculate on the mechanism behind this. It may be part of the "contraction" of scar tissue so often seen clinically. However, if this is true, why is such phagocytosis not seen more commonly in reparative processes?

A survey of the literature on the histologic aspects of healing gastric ulcers fails to reveal any specific comment on phagocytosis of collagen during the reparative phase of an ulcer. This may depend on the stage in the destructive or reparative period which the specimen represents.

Since the classic observations of Metchnikoff [9] on phagocytosis there have been innumerable studies on the subject. However, in a search for comparisons between the reparative processes seen in healing wounds and the mechanism of the recasting of the various body structures during fetal development, one finds little that has direct bearing on the problem.

The "intracellular digestive power" of isolated mesenchymal cells over dead protein was recorded by Danchakoff,[10] who concluded that they retain their digestive power over dead protein of their own kind from embryonic life.

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8. Foot, N. C., and Mènard, M. C.: Arch. Path. **4**:211, 1927.
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10. Danchakoff, V.: Anat. Rec. **18**:230, 1920; Am. J. Anat. **29**:431, 1921.

There seems to be no reason why one should not expect to observe phagocytosis of collagen as well as phagocytosis of many of the breakdown products formed in the base of a large peptic ulcer, the bed of which has undoubtedly been previously attacked and partly digested by pepsin and hydrochloric acid. Whether this process is destructive or reparative is entirely speculative.

SUMMARY

Phagocytosis of collagen in the bases of two gastric ulcers seems evident from the morphologic aspects and staining properties of the macrophages observed.

SPECIFICITY OF FETAL AND OF ADULT HUMAN HEMOGLOBIN PRECIPITINS

RUTH RENTER DARROW, M.D.

SOPHIE NOWAKOVSKY, M.D.
AND
MARGARET HOWARD AUSTIN, M.D.
CHICAGO

This study was undertaken to determine whether hemoglobin in the newborn human infant differs as an antigen from that of the human adult.

The species specificity of hemoglobin has been demonstrated by a number of investigators, using various methods. Körber,[1] in 1866, was the first to call attention to the different velocities with which the blood pigments from different animals were destroyed by alkalis and acids and to the constancy of the rate of such destruction for any one animal species. Von Krüger [2] corroborated these findings and established the fact of this specificity beyond question. The "von Krüger reaction" measures the rate of denaturation of an oxyhemoglobin solution to which one fifth of a volume of fourth-normal sodium hydroxide has been added, the time required for the disappearance of both absorption bands of oxyhemoglobin being observed by means of a spectroscope. Concerning this reaction, Haurowitz [3] commented, "The von Krüger reaction demonstrates in the simplest way the specificity of the hemoglobins of different animals. This specificity . . . manifests itself also in the crystalline form, the solubility, the serologic precipitation, and the affinity for oxygen or carbon monoxide."

Although differences in the crystalline forms of hemoglobins from various animal species were noted by Reichert and Brown,[4] the specificity of precipitin formation and even the antigenicity of hemoglobins long remained a moot question. Leblanc [5] in 1901 reported that in rabbits injections of hemoglobin give rise to precipitating antibodies in the serum. This report was confirmed by Ide [6] and by Demees.[7] The painstaking work of the latter, with hemoglobin solutions that

From the Women and Children's Hospital.

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5. Leblanc, A.: Cellule **18**:337, 1901.

6. Ide, M.: Cellule **20**:263, 1902.

7. Demees, O.: Cellule **24**:423, 1907.

were freed as completely as possible from serum and stroma proteins by repeated precipitation, demonstrated the specific precipitinogenic activity of injected hemoglobin and showed further that the "antihemoglobin" is specific for hemoglobin alone, since it does not react with the other proteins of the red cells or serum, does not hemolyze or otherwise affect the red cell and precipitates the specific hemoglobin only after the liberation of the latter from the erythrocyte. Subsequently Thomsen [8] and Bradley and Sansum,[9] utilizing the anaphylactic reaction, corroborated the reports of the antigenic effect of hemoglobin, and Klein,[10] Leers [11] and Fugiwara [12] confirmed the reports of its precipitinogenic activity. On the other hand, Ford and Halsey [13] were unable to show either precipitin formation or anaphylaxis by injecting hemoglobin that had been repeatedly recrystallized, and Schmidt and Bennett [14] obtained similar negative results. Since in many instances the solutions of hemoglobin used in obtaining the positive results were obviously not adequately freed from all other proteins, such results were not regarded as acceptable. On the other hand, the negative results reported may have been due to one or more of several causes. Hemoglobin is easily denatured, and the method used in its preparation, purification or storage may thus be responsible for loss of antigenicity. Furthermore, as pointed out by Hektoen and Schulhof,[15] sufficient dilution of the antigen is necessary in making tests for hemoglobin precipitins, since too concentrated a solution may cause inhibition of the reaction. The same investigators also called attention to the importance of using a number of animals for injection, as rabbits vary greatly in their ability to produce antibodies. Finally, as shown by Demees,[7] multiple injections are necessary for the production of hemoglobin precipitins. In 1923 Hektoen and Schulhof,[15] utilizing a method suggested by the work of Marshall and Welker,[16] employed colloidal aluminum hydroxide to obtain a hemoglobin solution from which all stroma and serum proteins had been removed by adsorption. By thus assuring adequate purity of the antigen, they conclusively demonstrated the precipitinogenic activity and specificity of hemoglobins. The same year Heidelberger and Landsteiner [17] and Higashi [18] obtained similar results with hemoglobins three times recrystallized. The latter, furthermore, confirmed the findings of Demees that hemoglobin antiserum contains neither hemolysins nor agglutinins. On the other hand, he found it to possess complement-fixing and anaphylactic antibodies as well as precipitins. He also observed the "organ specificity" and, except in the case of certain related groups, the species specificity of hemoglobin antiserums.

With the species differences in hemoglobins thus firmly established, first by the velocities of their denaturation by alkali and finally by immunologic methods,

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the observations of Körber [1] and later of Wakulenko [19] are of much interest. These investigators showed by means of the velocity of denaturation that the hemoglobin in the umbilical blood of the newborn infant differs from that of the adult human being. This finding has been confirmed by Bischoff,[20] Bischoff and Schulte,[21] Wundt,[22] Haurowitz,[3] Trought [23] and Brinkman and Jonxis.[24] The methods by means of which the marked difference in the speeds of reaction to alkali of the two human hemoglobins has been determined include the colorimetric method of Bischoff and Schulte, the spreading velocity technic of Brinkman and Jonxis and an analytic chemical method used by Haurowitz, as well as the spectroscopic method employed by Bischoff, Wakulenko and Trought. The distinction between the two hemoglobins has been further corroborated by Perrier and Jannelli,[25] who found definite differences in the crystals of neonatal and adult human hemoglobins.

An entirely different procedure differentiating fetal from adult hemoglobin in a number of species is that employed by Barcroft [26] and the workers in his laboratory. McCarthy [27] prepared from fetal and maternal goat bloods the corresponding hemoglobins in a condition approximating chemical purity at p_H 6.8. The oxygen dissociation curves of the hemoglobins so prepared showed distinct differences, the hemoglobin of the fetus taking up oxygen more readily than that of the mother. Hall [28] found similar differences. Barcroft concluded from these observations that "the characteristic dissociation curve of fetal blood is due to a specific form of hemoglobin." Eastman, Geiling and de Lawder,[29] however, although noting that fetal blood, both in its oxygen and in its carbon dioxide relationships, differs considerably from that of the adult, stated that "it seems probable that these differences can be explained on a strictly physico-chemical basis and that they are conditioned by those laws which govern the diffusion of these gases through a wet membrane." Leibson, Likhnitzky and Sax,[30] on the other hand, found that with equal hydrogen ion concentration the percentage of oxygen saturation in fetal blood considerably exceeds that in adult blood, and concluded, therefore, that "a dissociation curve lying higher and differing in shape from that of an adult leads us to suppose that the peculiar character of the oxyhemoglobin dissociation curve for fetal blood is really brought about by the presence of a 'fetal' form of hemoglobin in the blood of the fetus."

This distinction between the fetal and adult forms of hemoglobin has been indicated by the oxygen dissociation curves not only in the case of human [26] but in that of goat,[31] cow,[26] rabbit [28] and chicken [28] blood, according to Barcroft

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and his co-workers. For species other than the human the velocity of denaturation by alkali, on the other hand, has given negative or conflicting results. Hentschel [32] found no differences between newborn and maternal hemoglobin in dogs, horses and rabbits. Bischoff [33] detected no change in guinea pigs, cats, rats and chickens but found that in rabbits the resistance of the hemoglobin was less soon after birth than a few weeks later and also that the hemoglobin of the newborn rabbit was less resistant than that of the adult animal. Brinkman and Jonxis,[34] although reporting negative findings at first,[24] found later that while the hemoglobin of the fetal kid and calf is much less resistant than that of the adult animals, the hemoglobin of the fetal rabbit, like that of the fetal human being, surpasses in resistance that of the adult. These discrepancies are in marked contrast to the uniformity in the results of the investigations concerning the two forms of human hemoglobin.

Bischoff and Schulte [21] concluded from their observations that the hemoglobin in the blood of the human infant at term is a mixture of two distinctly different hemoglobins, one identical with or indistinguishable from that found in the adult, the denaturation of which by alkali occurs with great rapidity (in forty to seventy seconds), the other not found at all in adult human blood, the denaturation of which occurs very slowly over a period of hours. The latter form of hemoglobin is that designated as "fetal" human hemoglobin, and constitutes at term, according to Haurowitz,[3] about 80 per cent of the hemoglobin in the blood of the newborn infant. Haurowitz was able to effect a chemical separation of the fetal from the adult hemoglobin in the blood obtained from the umbilical vein at birth by treating the hemoglobin mixture with alkali for five minutes, neutralizing, and precipitating out the denatured adult hemoglobin by one-third saturation with ammonium sulfate in the presence of ammonium hydroxide, the undenatured "fetal" hemoglobin remaining largely in solution. Bischoff and Schulte [21] had arrived at similar conclusions as to the relative proportions of the two hemoglobins in newborn blood. They found, furthermore, that the transition in the hemoglobin of the infant from the fetal to the adult type occurred gradually, the proportion of fetal hemoglobin being greater the shorter the interval from birth and lessening rapidly with the increasing age of the infant until no longer present toward the end of the first year of life. Trought,[23] indeed, could detect no "fetal" hemoglobin in the blood of an infant of 4½ months, although it was still strongly present in that of an infant of 1 month. In a premature infant, on the other hand, in the study by Bischoff and Schulte, the proportion of fetal hemoglobin was found to be even greater than in the full term infant. These observations indicate a gradient for fetal hemoglobin, with maximal values at some time during fetal life, the proportion in the total hemoglobin already decreasing at the time of birth in the full term infant.

The difference between the fetal and the adult form of hemoglobin seems to relate to the globin portion of the respective hemoglobin molecules. Haurowitz [3] showed that while the prosthetic groups in fetal and adult human hemoglobin appeared to be identical, each behaving like alpha hemin, the globins of the respective hemoglobins reveal differences in denaturation time when treated with alkali, corresponding to those shown by the original hemoglobins. The role of the globin fraction in the production of specific precipitins for hemoglobins was

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tentatively set forth by Hektoen and Schulhof.[35] Their conclusions have been definitely established by Johnson and Bradley,[36] who showed that precipitins evoked by injections of the undenatured globins appear to be identical with those produced by the hemoglobins from which the globins were derived and that such globins are as species specific as the corresponding hemoglobins. They concluded that the globin is responsible for the antigenic properties of hemoglobin and that the species-specificity of the various globins is apparently due to differences inherent in their structure.

In view of all this extremely suggestive evidence indicating that human adult and fetal hemoglobins differ chemically from each other, that this difference resides principally in the globin portion of the molecule and that the globin fraction is responsible for the immunologic specificity of the hemoglobin molecule, it seemed worth while to determine whether this difference would be reflected in the production of specific precipitins when hemoglobins from human newborn and adult subjects, respectively, were injected into experimental animals. Wells [37] pointed out that it is the chemical character of a protein which determines its specificity rather than its biologic source. An investigation utilizing the immunologic method should, therefore, yield clearcut and conclusive evidence of any real distinction between fetal and adult human hemoglobins. The following procedures were therefore carried out to determine whether fetal human hemoglobin is antigenically distinct from adult human hemoglobin.

PROCEDURES

Preparation of Hemoglobin Antigens.—Pure oxyhemoglobin solutions were prepared from adult human blood and from blood obtained from the placental end of the umbilical vein of a full term newborn infant. The blood was collected into sterile flasks, with potassium oxalate as an anticoagulant. Ten cubic centimeter portions of each blood were transferred to sterile 50 cubic centimeter centrifuge tubes, sterile physiologic solution of sodium chloride added and the contents centrifuged. The cells were washed five times with sterile physiologic solution of sodium chloride, the supernatant fluid present after each centrifuging being discarded. The washed cells were then hemolyzed with three portions of 10 cc. each of chilled sterile distilled water, the resulting hemoglobin solution being removed each time, and the three portions finally pooled. The hemolysates were then each treated with 30 cc. of aluminum hydroxide cream, prepared as described by Boor and Hektoen [38] after the method of Marshall and Welker,[16] with care to preserve bacterial sterility. After thorough mixing, the solutions were filtered through paper in the refrigerator. This treatment with aluminum hydroxide cream was repeated three times, resulting in clear cherry-red solutions, which filtered rapidly. These solutions were then made up to the concentration of salt in physiologic solution of sodium chloride, stoppered tightly and kept in the refriger-

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36. Johnson, C. A., and Bradley, W. B.: J. Infect. Dis. **57**:70, 1935.

37. Wells, H. G.: J. Infect. Dis. **19**:183, 1916; Chemical Aspect of Immunity, ed. 2, New York, Chemical Catalog Company, Inc., 1929.

38. Boor, A. K., and Hektoen, L.: J. Infect. Dis. **46**:1, 1930.

ator. The concentration of the solutions was such that 20 cc. represented the hemoglobin derived from the erythrocytes in 1 cc. of blood.

Preparation of Antiserums.—Six intravenous injections of each prepared oxyhemoglobin antigen were made into rabbits at three day intervals, beginning with 2 cc. and increasing by 2 cc. each time, making a total minimum of 42 cc. over a period of sixteen days. Two rabbits were given injections of the hemoglobin prepared from adult blood. Two were given injections of hemoglobin prepared from blood of the umbilical cord. Five days after the last injection blood was withdrawn from each rabbit and the serum obtained.

Tests for Specific Precipitins.—Microprecipitin tubes were used, a small amount of antiserum being placed in each. The hemoglobin antigen was then introduced with a capillary pipet to form a supernatant layer, progressive dilutions of the antigens being used. The results were read after one hour at room temperature.

Positive precipitin reactions were obtained from the serums of 3 rabbits. One of the rabbits receiving adult hemoglobin showed no precipitins. All positive serums reacted with both hemoglobins.

Specific Absorption Tests.—Portions of both umbilical cord and adult hemoglobin antiserums were mixed with equal quantities of each hemoglobin solution, respectively. After thorough mixing, the four tubes were kept at room temperature for several hours and were then placed in the refrigerator over night. The resulting precipitates were removed by centrifugation, and the clear "absorbed" serums remaining were then each tested with fetal and adult hemoglobin solutions, respectively.

No further precipitation occurred in the absorbed serums with one exception. The cord hemoglobin antiserum which had been absorbed with adult hemoglobin gave a further positive reaction with cord hemoglobin but not with adult hemoglobin.

In these tentative preliminary experiments the results were definite qualitatively, but the titers were not high. The procedures were therefore repeated, fresh hemoglobin solutions being prepared from the following sources: (*a*) adult human blood, (*b*) cord blood of a full term infant, (*c*) cord blood of an infant born six weeks prematurely and (*d*) cord blood of an infant born two and a half months prematurely. The animals previously given injections were given further injections of the same antigens; 3 additional rabbits were given injections of adult hemoglobin, and 3, full term cord hemoglobin. The two hemoglobins prepared from the cord bloods of premature infants were each injected, respectively, into 2 rabbits. All the serums of the animals receiving reinjections gave high titers for both antigens, those of the 3 animals receiving injections of adult hemoglobin for the first time showed no precipitin response, while the serums of the 3 given injections of full term cord hemoglobin for the first time each gave a fair titer with both hemoglobins. Neither of the serums from the rabbits receiving the hemoglobin from the six week premature infant showed precipitin formation, while that of 1 of the animals receiving hemoglobin from the two and a half month premature infant yielded a strongly positive reaction with two and a half month premature cord hemoglobin, but a completely negative reaction with adult hemoglobin. Normal rabbit serum gave no reaction with any of the hemoglobin preparations.

Portions of the antiserums thus obtained for adult hemoglobin (second series of injections), full term cord hemoglobin (second series of injections), full term cord hemoglobin (first series of injections) and two and a half month premature cord hemoglobin were thoroughly mixed with, respectively, equal amounts of each

hemoglobin solution, allowed to stand at room temperature and then placed in the refrigerator over night. The following day the precipitates were removed by centrifugation, and each of the clear supernatant "absorbed" serums was tested for further precipitation with each of the hemoglobin antigens.

RESULTS

None of the absorbed adult hemoglobin antiserums showed further precipitation with any of the hemoglobin solutions in any dilution.

Both full term cord hemoglobin antiserums absorbed by adult hemoglobin showed negative precipitin reactions when tested with adult hemoglobin but gave strongly positive reactions with all of the cord hemoglobins.

Full term cord hemoglobin antiserums absorbed by full term or premature cord hemoglobins showed no further reaction with either adult or cord hemoglobins.

No precipitin reaction occurred in the absorbed two and a half month premature cord hemoglobin antiserums with any of the four hemoglobin solutions. Repetition of the titrations and of the absorption experiments with this antiserum gave results identical with those first obtained. The two and a half month premature cord hemoglobin solution was then tested against adult, full term cord and two and a half month premature cord hemoglobin antiserums simultaneously. With adult hemoglobin antiserum the titer was 1:16; with the two cord antiserums the titer was 1:512.[39]

COMMENT

If cord blood hemoglobin is a mixture of adult and fetal hemoglobin, it will show precipitation both with adult and cord blood hemoglobin antiserums, and, conversely, it will give rise to precipitins which will react with both adult and cord hemoglobin solutions. While both these expectations are fulfilled, these reactions are not conclusive that fetal and adult hemoglobins are antigens distinct from each other. The absorption tests, on the other hand, provide positive evidence of this distinction. Absorption of adult hemoglobin antiserum by adult hemoglobin or by cord hemoglobin, which presumably contains adult hemoglobin, removed all antibodies for adult hemoglobin, and no further precipitation occurred with either adult or cord hemoglobin solutions. Absorption of cord hemoglobin antiserum, which presumably contains antibodies for both adult and fetal hemoglobins, by cord hemoglobin, which is a mixture of fetal and adult hemoglobins, removed all the anti-

39. These figures represent the titer of the antiserum with the hemoglobin solution as prepared. No quantitative determinations of the hemoglobin in these solutions were made, as qualitative results only were being sought. It may be stated, however, that the hemoglobin content of our solutions was probably less than 0.5 per cent.

bodies for both antigens and no further precipitation occurred with either antigen. On the other hand, cord hemoglobin antiserum, containing antibodies for fetal hemoglobin which were not removed by absorption with adult hemoglobin, showed further precipitation as the result of the interaction between the remaining antibodies for fetal hemoglobin and the cord hemoglobin containing fetal hemoglobin. The discrepancy in the case of the antiserum for two and a half month premature cord hemoglobin merits further consideration, but speculation as to its cause is at this time, and without further or repeated investigation, hardly justifiable. It does not, however, invalidate in the least the positive evidence that more than one specific hemoglobin is to be found in the human species, for while the titer of the two and a half month premature hemoglobin antiserum was relatively high for the corresponding premature hemoglobin, no precipitin reaction was obtained with adult hemoglobin. Conversely, while the two and a half month premature cord hemoglobin showed a low titer with adult hemoglobin antiserum, indicating a small admixture of adult hemoglobin, the titer with full term and two and a half month premature hemoglobin antiserums, both of which are high in precipitins for fetal hemoglobin, was many times larger. These experiments, therefore, corroborate the work of other investigators, showing conclusively that in the blood of the newborn infant there is a mixture of at least two distinct hemoglobins, one of which is no longer to be found in the blood of the human adult.

SUMMARY

Antiserums evoked by hemoglobin prepared from the umbilical cord blood of the newborn human infant show positive precipitin reactions with both adult and cord human hemoglobins. After absorption with adult hemoglobin, further precipitation occurs with cord hemoglobin but not with adult hemoglobin. After absorption with cord hemoglobin, however, no further precipitation occurs with either hemoglobin.

Antiserums prepared by injection of adult hemoglobin show no further precipitin reaction with either adult or cord hemoglobin after absorption with either hemoglobin.

These observations must be interpreted as showing conclusively (1) that in the human species the hemoglobin in the blood of the umbilical cord at birth represents a mixture of at least two different and distinct hemoglobins, which may be designated as fetal and as adult hemoglobin, respectively, and (2) that fetal human hemoglobin is not demonstrable, by the immunologic method, in adult human blood.

MICROSCOPIC STRUCTURE OF STRIATED MUSCLE IN HEAT RIGOR

THE NODAL MULTIPLICATION OF STRIAE

EBEN J. CAREY, M.D.
MILWAUKEE

The graded microscopic changes of the structure of striated muscle suddenly and directly stimulated and immediately fixed in situ during different degrees of isometric heat rigor have not been recorded in the literature.

Fletcher and Hopkins [1] found that frog muscles after heat rigor yield as much as 0.5 per cent by weight of zinc lactate, or about 0.35 per cent of lactic acid, which corresponds to about twenty-fifth normal solution. Wells [2] used solutions of lactic acid of thirty-second normal strength to produce microscopic changes in striated muscle in vitro and in vivo resembling Zenker's waxy degeneration. He claimed that defective circulation in injured muscle causes the structural change. Smith [3] studied the heat produced during rigor of mammalian muscle and concluded that stiffening and lactic acid production from the breakdown of glycogen are associated with a third related chemical factor. Smith suggested that this may be either hydrolysis of creatinine phosphoric acid or a still unknown chemical change, as suggested by Meyerhof, McCullagh and Schulz [4] and by Lundsgaard.[5]

Zenker [6] observed microscopically the hyaline degeneration of voluntary muscle found in fatal typhoid fever in Dresden from 1859 to 1862. Forbus [7] studied the degeneration and regeneration of the human rectus abdominis muscle in the fall of 1918, during the severe influenza epidemic in the United States Army camps. Forbus concluded that the cause of

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From the Department of Anatomy, Marquette University School of Medicine.

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7. Forbus, W. D.: Arch. Path. **2**: (*a*) 318 and (*b*) 486, 1926.

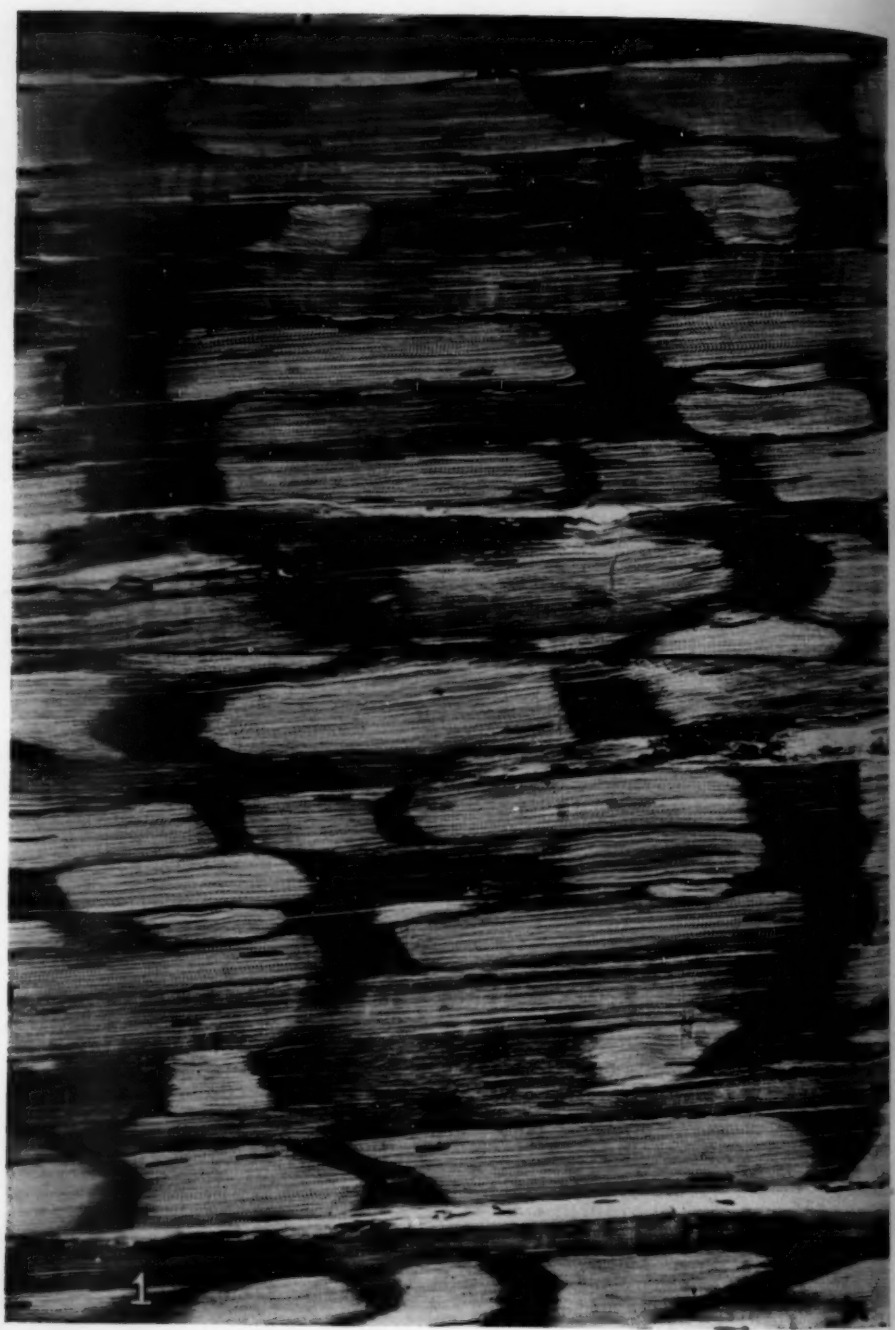

Figure 1

(See legend on opposite page)

the muscle degeneration is unknown, although the evidence indicates that a bacterial toxin is responsible. He found no evidence pointing to a localized infection in the muscle. The degree of muscle degeneration parallels the intensity of the infection and not its duration. Furthermore, the lesion was not found to be dependent on pneumonia due to any specific type of organism. Weihl [8] and Thoma [9] found that the injured muscle fibers of the tongue of the living frog may undergo what they call a form of waxy degeneration while being examined under the microscope. Thoma concluded that the silver gray appearance of the waxy muscle fibers is due to the phenomena of interference and refraction caused by the compact striations.

The production of fixed and traveling nodes of contraction in striated muscle comparable to those in smooth muscle was studied by Bowman,[10] Exner,[11] Rollett,[12] Heidenhain,[13] Hürthle,[14] Ernst and Kellner,[15] Nageotte,[16] Speidel,[17] Meneely [18] and many others. These nodes are reversible or irreversible and may either remain fixed in situ or travel by involving the entire cross-sectional area or part of the fiber. The physical significance, however, of the microscopic nodes and internodes in striated muscle is unknown.

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Explanation of Figure 1

Photomicrographs (untouched) of the rectus abdominis muscle of the frog (Rana pipiens); longitudinal section; 8 microns; × 150; hemalum and eosin. The muscle has been subjected to a sudden elevation of temperature to 37 C. for ten seconds, then to quick cooling and fixation. Note transverse and oblique pressure nodes of heat contracture of the complete and incomplete types, darkly stained, containing fine, closely spaced striations and dark rounded nuclei. The tension internodes are lightly stained and have coarse, widely spaced striae and nuclei two to three times the length and one-half the diameter of the nodal nuclei. The nodes and internodes are the components of the isometric contraction compression waves associated with extreme chemical changes in a microcapillary tubular muscle fiber.

FIGURES 2-3-4

(See legend on opposite page)

The most difficult microscopic problem to solve is the exact change of striae when nodes of strong contraction appear in striated muscle. Both Speidel and Meneely recognized this difficulty in observing the structural changes in living muscle fibers. Speidel [17b] observed a blurring of striations just before the stage of formation of so-called contraction bands. Meneely [18] stated that when a node formed, the striations were pressed together so closely that it was difficult to make them out.

An attempt will be made to answer the following question in this paper:

Are the fine striae of the node due to a passive approximation of the coarse, relatively resting ones, or are they a new set of fine striae replacing the resting ones but continuous with those of the internode?

In the present state of knowledge and confusion regarding muscle striae the photomicrographic method is superior to that of free hand drawings of visual impressions. Since heat alters the rate of chemical reactions and at a critical temperature and duration produces rigor of muscle and favors the destructive rather than the constructive chemical changes, it was decided to study microscopically both the living and the fixed muscle after both direct and indirect heat stimulation through graded degrees of temperature. Only those observations will be presented in this paper which deal with the microscopic changes in the striated muscle of the winter frog following a short sudden rise of temperature to 37 C.

MATERIALS AND METHODS

The winter frog (Rana pipiens) was used in these experiments because the nuclei of its muscle fibers are more numerous and are found throughout the fibers of the rectus abdominis muscle. These nuclei serve as an index of the alternate zones of pressure and tension, the components of the longitudinal waves of compression associated with the altered chemical changes of sudden heat rigor of

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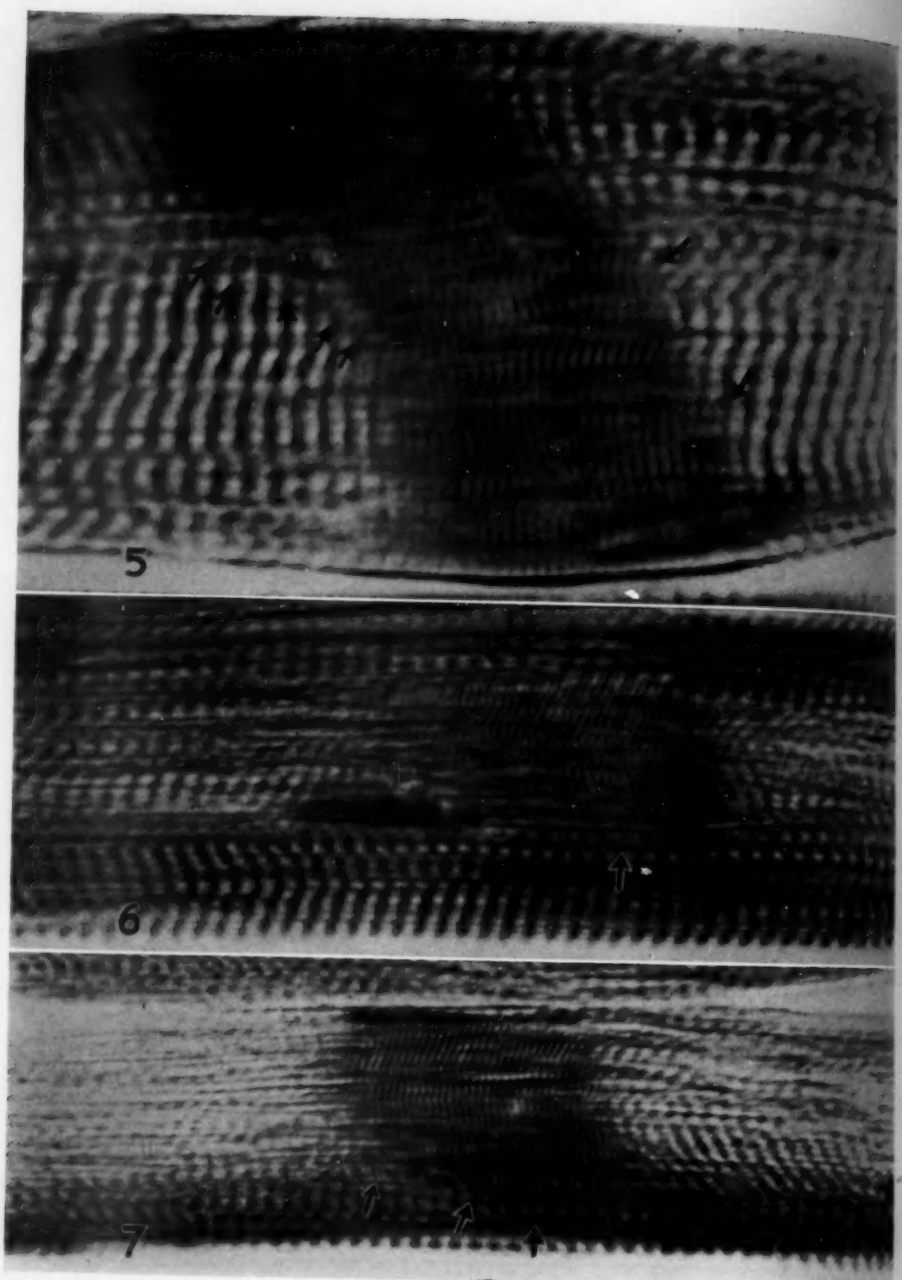
EXPLANATION OF FIGURES 2, 3, AND 4

Photomicrographs (untouched) of the rectus abdominis muscle of the frog (Rana pipiens); × 700, 1,000 and 1,000, respectively; longitudinal sections; 8 microns; iron hematoxylin and eosin. The muscle has been subjected to a sudden elevation in temperature to 37 C. for ten seconds, followed by quick cooling and fixation. Note transverse contracture nodes of reversible type in figures 2 and 3, with fine, uniformly spaced dark and light bands and oblong, compressed, deeply stained nuclei. The tension internode to the right in this same fiber has the striation pattern of Q, J, Z, J, Q and stretched granular nuclei twice the length of those in the pressure node. The obliquely placed node (fig. 3) is composed of fine striae which appear to be split products of the coarse, thick dark striae in the internode. This cleavage is best observed in higher magnifications (figs. 5, 6 and 7). There are no fine dark Z bands in the light J bands of the internode in this muscle fiber. The arrows indicate locations of splitting of some of the coarse into fine striations.



FIGURES 5-6-7

(See legend on opposite page)

short duration. Forty animals were used in this experiment. Each frog, unanesthetized, was tied to a glass plate with the lower limbs approximated to the midaxial line and extended. Two additional transverse cords were used to hold the frog flat to the plate, one over the midthoracic region and the other just below the pelvic girdle. The skin was dissected from the ventral aspect of both rectus muscles. The frog, held flat to the plate, was placed on the stage of the ultrapac microscope and the exposed muscle fibers observed with the water immersion lenses. When Locke's solution at 37 C. was placed on the muscle, many fine complete and incomplete peristaltic-like nodes appeared suddenly and traveled through the muscle fiber. When Locke's solution at 40 C. was used, these nodes become fixed and more opaque than at 37 C. I have had the same difficulty that both Speidel and Meneely recorded in deciphering the exact changes that occur in the living muscle fiber when coarse striae are replaced by fine striae. I do not believe this problem will be solved by a study of the living muscle. Even when the transition from coarse to fine nodal striae is a slow one it is still too complex a change to be studied adequately in the living muscle by either still or moving picture methods. Only the changes of the striae nearest the surface of the living muscle fiber may be seen clearly.

It was decided to fix the traveling peristaltic-like nodes generated at 37 C. in Locke's solution. Frogs tied flat to glass plates and prepared as outlined in the foregoing paragraph were dipped into Locke's solution at 37 C. for ten seconds and then quickly placed in Locke's solution at 4 C. for fifteen minutes to inhibit quickly the rate of chemical change. Two lateral openings were made in the abdominal cavity to allow the fixative to reach both surfaces of the rectus muscles, and then the frogs were placed either in Bouin's solution or in 10 per cent formaldehyde U. S. P. at 4 C. for six to ten hours. The intact muscles fixed

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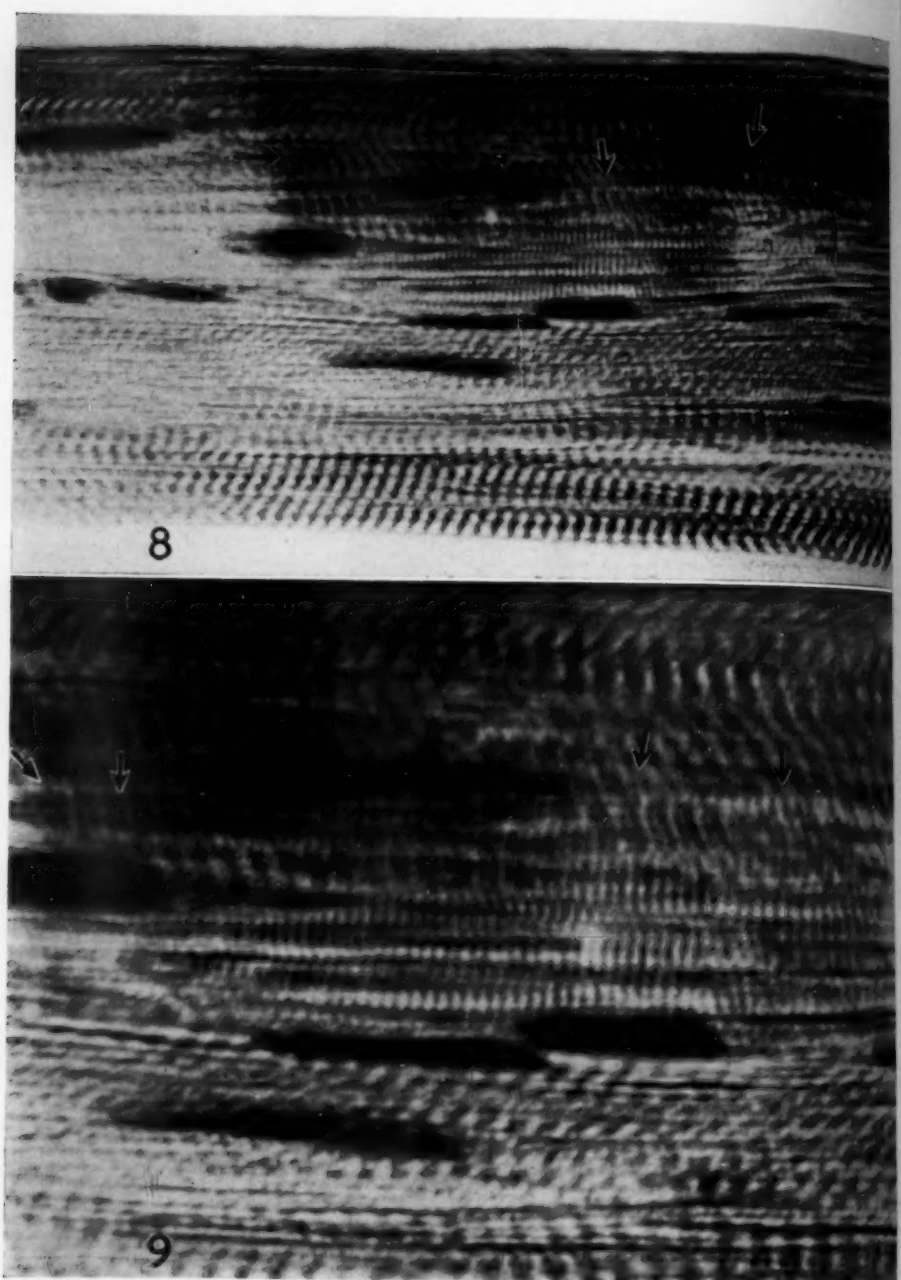
EXPLANATION OF FIGURES 5, 6 AND 7

Photomicrographs (untouched) of the rectus abdominis muscle of the frog (Rana pipiens) × 1,200, 1,000 and 1,000, respectively; longitudinal sections; 8 microns; iron hematoxylin and eosin. The muscle is in reversible heat rigor, 37 C. The contraction node (fig. 5) is obliquely placed to the direction of the long axis of the muscle fiber. Along the margins the thick, coarse dark striae of the noncontracted internode undergo cleavage or branching into two, three or four fine striations, which constitute those of the node. When the margin of the node is perpendicular to the long axis of the muscle fiber, this phenomenon of splitting or cleavage is not apparent. The multiplication of the coarse dark striae into the fine dark ones is also readily apparent when only part of the transverse sectional area is occupied by a partial node of contracture (figs. 6 and 7). The marginal coarse dark striae are observed to split into two fine dark bands. These are continuous with the uniformly spaced bands of the partial pressure node. In places what appears to be a dark Z band within the light J band is evident, and these are continuous with the finer bands of the contraction nodes. In places there appears to be a blurring along the zone of transition between the coarse and fine dark striations (figs. 5, 6 and 7). The division of the coarse internodal and relatively inactive dark bands into the finer nodal and active dark ones is better followed in serial sections than in isolated sections or in optical observations of either living or fixed whole muscle fibers. The arrows indicate locations of splitting of some of the coarse into the fine striations.



FIGURES 8-9

(See legend on opposite page)

in isometric contraction were excised and serially sectioned, some transversely, others longitudinally. Various stains were used, including iron hematoxylin and eosin, and hemalum and erythrosin. The latter stain is better than the former for the nuclei.

MICROSCOPIC OBSERVATIONS

The alternate darkly stained nodes and lightly stained internodes may be observed in figure 1 of the rectus abdominis muscle of a frog fixed after the muscle had been stimulated in Locke's solution at 37 C. for ten seconds. In this low power view the compact fine striae that constitute the darkly stained nodes are invisible, but the pyknotic rounded nuclei are apparent. In the internodes coarse striae are visible as well as the elongated granular nuclei. The fine nodal and coarse internodal striae are shown under high power in figures 2, 3 and 4. In figures 2 and 3 the Z bands in the internodes are observed. The node is obliquely placed to the long axis of the muscle fiber (fig. 4). There is an apparent splitting of each Q stria of the internode into two to four finer striae of the node. To the right of the internode there is a doubling of the Q striae. There is no fine dark Z band in each J band of the fiber (fig. 4).

When the node is obliquely placed to the long axis of the muscle fiber or when it occupies only a part of the cross-sectional area of the fiber, the relationship of its component striae to those of the internode and noncontracted striae is more evident. The high power magnifications in figures 5, 6 and 7 give evidence that along the edges of the nodes the fine striae are the result of the splitting of the coarse ones of the internode. In some places there is direct continuity of one coarse internodal with one fine nodal stria. In others a division of one coarse stria into two, three and four fine striae is clearly observed (fig. 5). The products of division of coarse into fine striae may be on different planes. This results in blurring along the edges between the node and the internode. Single microscopic sections are not as favorable, therefore, as serial sections to observe the splitting of coarse into fine striations. Serial

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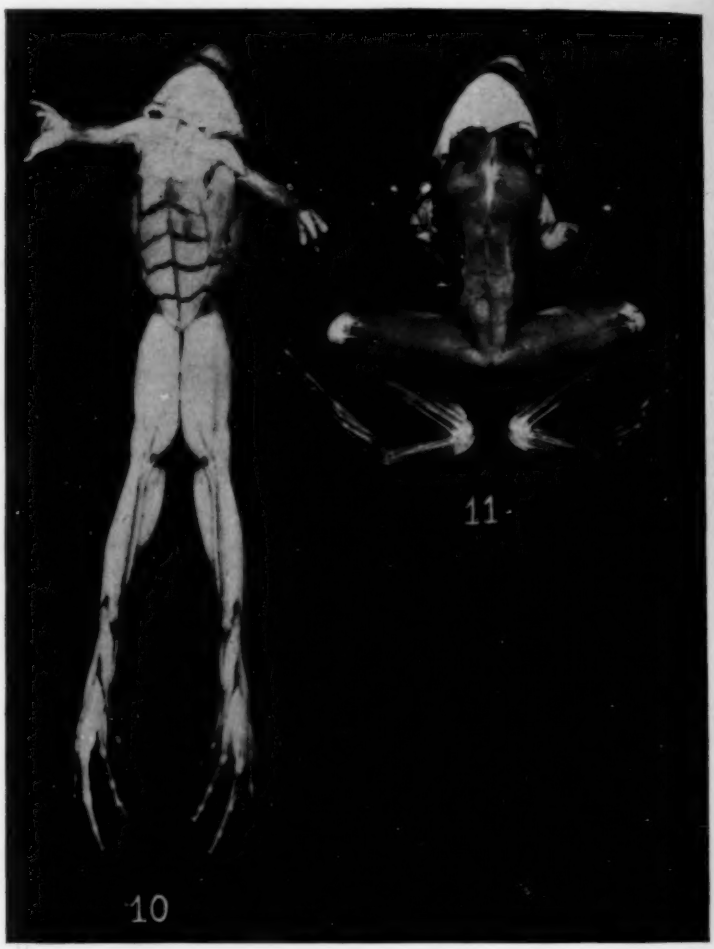
EXPLANATION OF FIGURES 8 AND 9

Photomicrographs (untouched) of the rectus abdominis muscle of the frog (Rana pipiens); × 700 and 1,200, respectively; longitudinal sections; 8 microns; hemalum and erythrosin. The muscle is in reversible heat rigor, 37 C. The partial contraction nodes of fine, uniformly spaced striae are continuous with the coarse dark striae of the uncontracted internodal region in the same muscle fiber. This cleavage of coarse into fine striae is microscopic evidence for the active multiplication of new fine nodal striae and against that of passive mechanical crowding of previously existing noncontracted striae of the relatively resting state. The arrows indicate locations of splitting of some of the coarse into the fine striations.

sections are better than either living or fixed muscle fibers to observe the phenomenon of the splitting of internodal into nodal striae. Along the upper (fig. 6) and the lower edge (fig. 7) of the incomplete contraction nodes the coarse Q split into two fine dark striae. This cleavage of the Q into two semidisks of Q has been emphasized by Jordan [19] in



Figs. 10 and 11.—When the skinned frog (fig. 10) is placed in Locke's solution at 45 C. for ten seconds there are a sudden extension of the limb and an opisthotonos position of the back comparable to that following a lethal subcutaneous injection of strychnine sulfate. The muscles become rigid and opaque. The inscriptiones tendineae between the segments of the recti abdomini muscles become prominent. The white and bloodless muscles in the extended position after heat rigor (fig. 10) are compared with the relatively dark muscles in the flexed position of the skinned frog at 18 C.

19. Jordan, H. E.: Physiol. Rev. **13**:301, 1933.

normal contraction of muscle. In serial sections this fact is still more apparent than in isolated, single sections. Furthermore, the cleavage is better observed in serial sections than in the entire muscle fibers in the fixed or the living state. The node has a more active multiplication of finer striae than has the continuously related internodal zone in the same muscle fiber.

The cleavage or multiplication of striae is also clearly observed (figs. 8 and 9) in regions of incomplete nodal formation in a muscle fiber. The relatively resting coarse striae may be seen in the upper and lower parts of each illustration. The apparent splitting of the continuously coarse into the finer striations is observed in the middle of the figures between the nuclei. Observations during the variations of the focus and on serial sections demonstrate that the splitting of coarse into fine striations is not an artefact due to superposition of striations. The partial contraction waves that occupy only a part of the cross sectional area and longitudinal length of the muscle fiber are especially favorable for observing the splitting of coarse into fine striae.

The nodes are strongly anisotropic and the internodes relatively less so or almost isotropic (Carey and Zeit [20]) in the contraction compression waves induced by heat, within physiologic limits, in active skeletal, transitional gizzard and smooth intestinal muscle. There is a residue of ash-bearing node and a relatively ash-free internode after microincineration (Carey and Zeit [20]) in the active skeletal, transitional gizzard and smooth intestinal muscles. The microincineration is done by Scott's [21] method. The distribution of mineral ash into serial nodes and internodes in all types of muscles activated by heat appears to be the same. The nodes are variably spaced, depending on the temperature and its duration. The mineral salts are easily and quickly mobilized into nodes throughout the muscle fiber and fibril and smooth muscle layers. There does not appear to be artificially fixed membranous barriers across striated muscle fibers that would delay the mobilization of inorganic salts. The so-called sarcomere with telophragmas of Z membranes is not a constant unit of structure in striated muscle. When the Z band is observed, it represents a certain level of muscle activity, based on an underlying chemical change. The histologic evidence on heat rigor presented in this paper agrees with the definition of muscle contracture based on physiologic findings presented in an extensive review by Gasser,[22] in which he stated that some part of the normal contractile mechanism must be involved and that the changes are reversible. The reversibility or irreversibility of the changes of the striae is dependent on the intensity and duration of the temperature.

20. Carey, E. J., and Zeit, W.: Proc. Soc. Exper. Biol. & Med. **41**:31, 1939.

21. Scott, G. H.: Proc. Soc. Exper. Biol. & Med. **29**:349, 1932; Am. J. Anat. **53**:243, 1933.

22. Gasser, H. S.: Physiol. Rev. **10**:35, 1930.

SUMMARY

Heat rigor of short duration is microscopically characterized by nodes and internodes, the component parts of strong contraction compression waves, in smooth intestinal, transitional gizzard and striated muscle in isometric contracture. The pressure nodes of the striated fiber contraction waves have deep differential chemical staining, wide diameter, condensed cytoplasm and rounded, compressed nuclei, multiplication of fine striae and an increase of anisotropy and mineral ash content. The tension internodes stain relatively less deeply and have narrow diameters, rarefied fibrillated cytoplasm, greatly elongated nuclei, coarse striae and a decrease of anisotropy and mineral ash content. The multiplication of nodal striae is best observed in nodes placed obliquely to the long axis of the muscle fiber and in partial contraction nodes of the muscle fiber. In the obliquely placed and partial contraction nodes the cleavage of the coarse, relatively inactive internodal striation into two, three or four fine active nodal striations is microscopically evident. Substantial histologic evidence appears to be presented that warrants the conclusion that the contraction node of heat rigor is not the result of a mere mechanical approximation of previously noncontracted striations but represents active multiplication of new fine and closely placed striae continuous with the coarse, inactive ones. There are variable degrees of muscle twitches, contraction and contracture with and without increase in the number of striations. The homogeneous nodes of intense heat rigor resemble those of Zenker's degeneration accompanying the fever of typhoid and pneumonia. Altered chemical changes in the muscle fiber appear to underlie and to be associated with the structural variations identified in heat rigor or contracture. The strong contraction compression waves of nodes and internodes associated with the chemical changes are oriented and alined into dark and light bands of compression and rarefaction by the microcapillary influence of the striated muscle fibers or fibrils.

MITOSIS IN SPECIMENS REMOVED DURING DAY AND NIGHT FROM CARCINOMA OF LARGE INTESTINE

WILLIAM B. DUBLIN, M.D.*

ROBERT O. GREGG, M.D.†

AND

ALBERT C. BRODERS, M.D.

ROCHESTER, MINN.

Evidence is accumulating which indicates that there is a daily rhythm of mitosis in normal animal tissues. Several investigators have found such a rhythm in newborn and young mammals.[1] Cooper and Schiff [2] determined the rate of mitosis in foreskins removed during the morning and night from infants 1 week old, and found approximately twice as many mitotic figures in specimens removed at night as in those taken during the day. Two of us (Broders and Dublin [3]), using slightly different histologic technic, were able to confirm this observation, and continued study by Cooper and Schiff has resulted in further confirmation. As yet, direct proof of the existence of a daily rhythm of mitosis in the adult is lacking. In normal adult tissues, mitoses are found so infrequently that their accurate quantitative evaluation is surrounded with great difficulty.

That a daily rhythm of mitosis occurs in the infant is surprising; for the cycle of activity of the newborn ordinarily is thought of in terms of four to six hours. The implication is strong that in the infant certain processes are maintained in accordance with "habits" which are formed while the fetus is still an appendage of the maternal body. This suggests that the same rhythm of mitosis which is found in the newborn occurs in the mother.

Daily rhythm of mitosis in the human body has not been explained adequately. It is possible that activity of mitosis is, roughly, inversely proportional to metabolic activity. There is no evidence, however, that mitosis responds to changes of short duration of the metabolic rate.

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* Fellow in Pathology, The Mayo Foundation.

† Fellow in Surgery, The Mayo Foundation.

From the Division of Surgical Pathology of the Mayo Clinic.

1. Carleton, A.: J. Anat. **68**:251, 1934.

2. Cooper, Z. K., and Schiff, A.: Proc. Soc. Exper. Biol. & Med. **39**:323, 1938.

3. Broders, A. C., and Dublin, W. B.: Proc. Staff Meet., Mayo Clin. **14**:423, 1939.

A possible explanation is that during the day, when the body is concerned chiefly with locomotion, the ingestion and digestion of food, and other processes of like nature which require the working together of many cells, and which in general are cytoplasmic activities, mitosis is at low ebb. During the night, when metabolism tends to fall to a basal level, and the body can afford to spare energy previously absorbed in the interfunctioning of cells and tissues, that energy is used in the formation of new cells (a nuclear activity), i. e., in repairing broken-down tissues and building new ones.

From an investigation of mitosis in normal, fully differentiated tissues one comes naturally to a consideration of the same problem in neoplasms. Aside from purely theoretic interest, certain factors, however fantastic, give a practical value to such a study. For instance, it is a well established principle that cells are most sensitive to radiation when in mitosis.

Mitosis Coefficients in Specimens Removed During Morning and Night

Case	Mitosis Coefficient		Adenocarcinoma, Grade	Situation
	A. M.	P. M.		
1	6.1	5.9	2	Colonic stoma
2	8.8	9.0	2	Colonic stoma
3	6.6	17.1	2	Rectum
4	8.9	7.9	1 (high)	Rectum
5	7.2	7.7	1 (high)	Rectum

MATERIAL

Five cases of carcinoma of the large intestine form the material of the present study. In cases 1 and 2, recurrences had occurred in the colonic stoma subsequent to resection. Two specimens were removed from each growth: one between 10 a. m. and 12 m., and one between 10 and 12 p. m. of the same day. In cases 3, 4 and 5, of polypoid carcinoma situated low in the rectum, biopsy specimens were removed from the polyps, between 10 and 12 p. m. the night before operation. The growths were removed between 10 a. m. and 12 m. the next morning, and sections were made from the operative specimens. The preoperative medication consisted of pentobarbital sodium and morphine. These drugs are not known to have an effect on mitotic division. Tissues were fixed without delay in 4 per cent solution of formaldehyde, sectioned on the freezing microtome and stained with hematoxylin and eosin. The mitosis coefficient, which is the best available index of the activity of cellular division, was determined for each specimen by recording the number of mitotic figures per thousand tumor cells. In each case, 3,000 to 5,000 cells were counted. The results are shown in the table.

OBSERVATIONS AND COMMENT

All growths were adenocarcinoma, grade 1 or 2 on a basis of 1 to 4. Specimens taken during the morning and night showed the same glandular pattern and degree of differentiation. The sections studied

contained, on the whole, a surprising number of mitotic figures, considering the grade of malignancy, and one section contained abnormal mitotic figures.

One specimen (case 3, p. m.) showed an area of relatively intense mitosis, out of proportion to that of the morning specimen and to those of specimens of the other tumors; this resulted in an unduly high coefficient for the specimen. It is not likely that this represented a change in the rate of mitosis of the growth. In sections from all other specimens the mitosis coefficients were essentially the same for morning and night, indicating the absence of a rhythm of mitosis.

This finding is not unexpected. Malignant cells grow without respect for the geographic boundaries which are set for normal cells. One may expect them also to grow with no consideration for the law of rhythm which one has reason to believe governs normal tissues; that is, one may expect them to grow at a constant rate.

This conclusion may apply to all malignant neoplasms. Direct proof of this supposition, however, may not be quickly forthcoming; for some tumor types, such as glioma, are at times so pleomorphic that a significant determination of mitosis is made with great difficulty. The aforementioned 5 cases of carcinoma were chosen because of the relative accessibility of the growths and the expected tendency to present a generally uniform type of tissue. It will be of interest to see if further study will, as has been the case with the epidermis of the infant, support these preliminary observations.

CHEMOTAXIS OF MONOCYTES CONTRASTED WITH THAT OF POLYMORPHONUCLEAR LEUKOCYTES AND LYMPHOCYTES

DALE REX COMAN, M.D.
PHILADELPHIA

Of the three types of leukocytes found in inflammatory exudates, polymorphonuclears, monocytes and lymphocytes, the polymorphonuclears are first to appear in an area of inflammation, but the monocytes are no less important in combating infections and in removing debris through their phagocytic activity. How are large numbers of these mononuclear phagocytes mobilized? Does this occur by means of the same mechanism that operates in the case of polymorphonuclears, namely, chemotaxis? Polymorphonuclears are known to be chemotactically attracted by most or all bacteria.[1] That a similar mechanism operates in the case of the monocyte has perhaps been assumed, but it has never been determined in vitro whether or not this cell actually responds to chemotactic influences. On the other hand, monocytes may in this respect resemble lymphocytes, which are reported [2] not to react chemotactically to bacteria, although these cells also accumulate in large numbers in areas of inflammation.

In view of these facts it seemed of interest to find out whether monocytes display chemotaxis, comparing their behavior in this respect with that of polymorphonuclears and lymphocytes under the same experimental conditions.

METHOD

In the experiments to be described tissue cultures were used, the technic for making which was obtained from Margaret R. and Warren H. Lewis, of Baltimore. In order to study the reactions of each of the three types of leukocytes, it was desirable to use explants which would yield the type of cell desired in almost pure culture. To obtain monocytes, small pieces of rat omentum were used, since during the first one or two days of incubation the migrating cells are almost exclusively mononuclear phagocytes. The polymorphonuclear preparations were made from the buffy coat of centrifuged blood of white rats. The lymphocyte preparations consisted of explants of rat axillary lymph node. In order to

From the Department of Pathology of the University of Pennsylvania Medical School.

This investigation was aided by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1. McCutcheon, M., and Dixon, H. M.: Arch. Path. **21**:749, 1936.
2. Dixon, H. M., and McCutcheon, M.: Arch. Path. **19**:679, 1935.

be certain as to the type of cell emigrating from these various explants, some of the preparations were fixed in solution of formaldehyde U. S. P. and stained with azure II-eosin, Giemsa's stain and hematoxylin and eosin.

The tissue to be explanted was transferred to Locke's solution and cut into tiny fragments about 0.5 mm. in diameter. The substance to be tested for its chemotactic effect was placed as a small clump, 1 mm. in diameter, on a cover slip. One or more pieces of tissue were transferred to this cover slip and placed in a position about 0.5 mm. from the clump of test substance. A thin film of heparinized autoplasma was added and the preparation, sealed to prevent evaporation, was incubated at 37 C. for eighteen to twenty hours. After this time the distribution of the emigrated cells in relation to the test material was recorded photographically and by camera lucida drawings.

OBSERVATIONS

Positive chemotaxis is exemplified in figure 1 *A*, a photograph which shows the reaction of polymorphonuclear leukocytes to Staphylococcus aureus. The explant is the oval mass in the center, and the bacteria

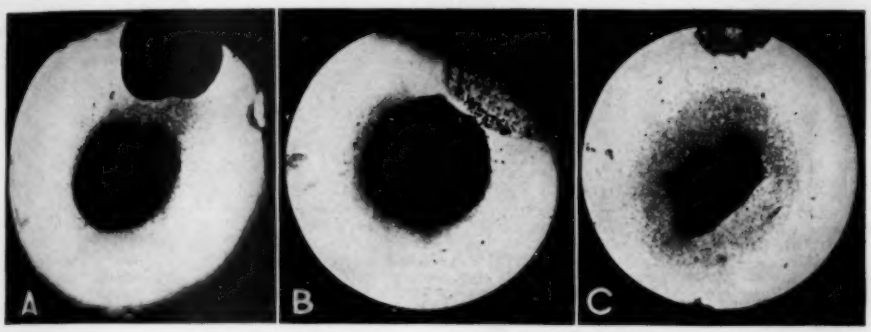

Fig. 1.—These photomicrographs are of tissue fragments from white rats as cultured in autoplasma, and show the chemotactic responses of the emigrating cells to a test substance.

A, positive chemotaxis. The central dark oval mass is the explant, in this instance a fragment of buffy coat. The dark peripheral mass above represents a clump of Staph. aureus. The emigrating polymorphonuclears form a hazy zone about the explant, this zone being much wider between the explant and the bacteria than elsewhere.

B, negative chemotaxis. The dark central mass is an explant of buffy coat. The peripheral stippled area above and to the right is aluminum silicate. The migrating polymorphonuclears form a hazy zone around the explant except on the side nearest to the silicate, where no cells occur.

C, indifferent chemotaxis. The dark central mass is an explant of omentum. The peripheral mass above is a clump of Staph. aureus. The emigrating cells are monocytes, and they form a broad zone of evenly distributed cells around the explant.

are represented by the dark peripheral mass above. The emigrating leukocytes form a hazy zone about the explant, a zone which is much wider between explant and bacteria than it is elsewhere.

Negative chemotaxis is shown in figure 1 *B*, which represents the repelling effect of aluminum silicate [3] on polymorphonuclear leukocytes. The silicate used was Lloyd's reagent, a refined fuller's earth. The explant is the dark circular mass in the center and the silicate is the stippled light and dark area above and to the right. No migrating cells can be distinguished in the direction of the silicate, but a broad zone of migration is seen on the opposite side of the explant.

Absence of chemotaxis (or indifferent chemotaxis) is exemplified in figure 1 *C*, which shows a broad zone of cells evenly distributed about the explant. This explant was a piece of omentum, the migrating cells monocytes, and the test substance a clump of Staph. aureus.

TABLE 1.—*Chemotactic Responses of Leukocytes* *

	Polymorphonuclears (Explants of Buffy Coat)			Monocytes (Explants of Omentum)			Lymphocytes (Explants of Lymp Node)		
	+	±	−	+	±	−	+	±	−
Staph. aureus	27	1	1	5	7	7	11	16	13
Tubercle bacilli (Bovine III)	31	9	6	8	18	9	13	11	8
Aluminum silicate	0	1	23	4	9	12	14	5	13

* The number of explants in which the migrating cells showed positive (+), indifferent (±) or negative (−) chemotaxis to three test substances are tabulated. The observations were made after about eighteen hours' incubation. Polymorphonuclears were obviously attracted by Staph. aureus and tubercle bacilli and were repelled by the silicate. No clearly defined positive or negative chemotactic reaction was shown by the monocytes and lymphocytes.

The results obtained with 282 such explants are given in table 1. This table is based on camera lucida drawings of the zones of emigration. A + sign indicates those explants in which the zone of migration was distinctly wider toward the test substance; a — sign, the preparations in which the zone of emigration was obviously broader on the side of the explant farthest away from the test substance, and a ± sign, those explants in which there was no distinct difference in the width of the zone of migration.

It is seen that in 27 of 29 explants the polymorphonuclears showed positive chemotaxis to Staph. aureus and that in 31 of 46 explants they showed a positive reaction to tubercle bacilli but that to the silicate they showed negative chemotaxis in 23 of 24 explants. These results with polymorphonuclears are in agreement with the results obtained by other methods.[4]

3. McCutcheon, M.; Coman, D. R., and Dixon, H. M.: Arch. Path. **27**:61, 1939.

4. McCutcheon and Dixon.[1] McCutcheon, Coman and Dixon.[3]

In contrast to the clearcut positive reaction to bacteria and negative reaction to silicate shown by the polymorphonuclears are the indifferent or doubtful reactions shown by monocytes and lymphocytes to these same substances. As shown in table 1, there is no clearcut example of positive or negative reaction by either monocytes or lymphocytes.

In order to determine whether these cells are actually indifferent to the test substances, further analysis of the data was necessary. For this analysis, camera lucida drawings were found more suitable than photographs. As described under figure 2, a value of chemotaxis for each explant was computed from the areas occupied by the migrating cells.

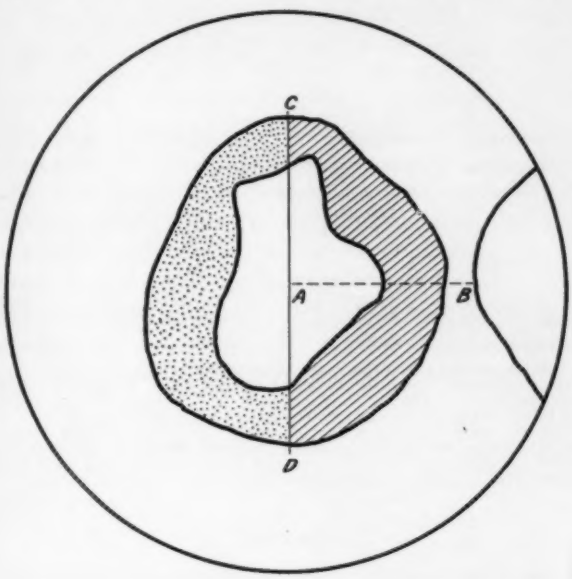

Fig. 2.—This is a camera lucida drawing of a microscopic field containing an explant in the center and a test substance at the periphery. The shaded and stippled areas represent the zone occupied by cells which have migrated from the explant. To determine the chemotactic value of this emigration zone, a line is drawn from the center of the explant (A) to the nearest point on the periphery of the test substance (B). A perpendicular to this line is constructed ($C\,D$), passing through the center of the explant and extending on each side to the limits of the migration zone. This line divides the migration zone into two parts, a positive portion (shaded area) and a negative portion (stippled area). The area of each of these portions is measured by a planimeter. The algebraic excess of the positive over the negative area is determined and is expressed as a fraction of the total area. In the original record of the example given, the positive area measured 3.8 square inches (24.6 sq. cm.) and the negative area 3.3 square inches (21.2 sq. cm.). The total area is 7.1 square inches (45.7 sq. cm.). The algebraic excess of the positive over the negative area is $+0.5$ square inches (3.2 sq. cm.) and the ratio of this value to the total area is $+0.070$, the value of chemotaxis.

The migration zone was divided into two parts, a positive portion facing the test substance (shaded area in fig. 2) and a negative portion (stippled area in fig. 2). The areas of the positive portion and of the negative portion were found by a planimeter. The algebraic excess of the positive over the negative area was determined and expressed as a fraction of the total area. This calculation was made for every explant, and with the data so obtained, the mean and standard error were determined for each kind of leukocyte with each kind of test substance.

The results appear in table 2 and confirm in general those given in table 1, polymorphonuclears being strongly attracted by both kinds of bacteria and strongly repelled by the silicate. However, it now appears that the monocytes were slightly repelled by the silicate, though they remained indifferent to the bacteria. Lymphocytes showed no chemotactic response.

TABLE 2.—*Mathematical Evaluation of Chemotactic Responses of Leukocytes**

	Polymorphonuclears (Explants of Buffy Coat)	Monocytes (Explants of Omentum)	Lymphocytes (Explants of Lymp Node)
Staph. aureus	+0.216 ± 0.038	+0.036 ± 0.042	−0.022 ± 0.046
Tuberculosis	+0.192 ± 0.034	−0.034 ± 0.024	+0.082 ± 0.058
Aluminum silicate	−0.472 ± 0.036	−0.115 ± 0.048	−0.034 ± 0.060

* Each number represents the mean value of chemotaxis with its standard error, as determined by analysis of camera lucida drawings of the explants. Unless the value of the mean is at least twice the value of the standard error, it is not regarded as significantly different from zero (indifferent chemotaxis). Maximal positive chemotaxis would have a value of +1 and maximal negative chemotaxis a value of −1. Thus it is seen that polymorphonuclears showed positive chemotaxis to Staph. aureus and tubercle bacilli and exhibited negative chemotaxis to the silicate. The monocytes were repelled by the silicate and were indifferent to the bacteria. Lymphocytes showed no chemotactic response to the test material.

COMMENT

Thus it was demonstrated that polymorphonuclear leukocytes were attracted by both Staph. aureus and tubercle bacilli and that they were repelled by aluminum silica. In contrast to the polymorphonuclears, the monocytes and lymphocytes, under similar conditions, failed to show positive chemotaxis to either of the two organisms. The monocytes, however, showed slight negative chemotaxis to the aluminum silicate.

It is concluded from these experiments that the monocyte, lymphocyte and polymorphonuclear leukocyte differ in their chemotactic reactions. Most striking is the failure of the monocyte to be attracted by bacteria, although it was observed that when this cell came into contact with the organisms, phagocytosis ensued.

In view of these results how can one account for the mobilization of monocytes in inflammatory foci? It is possible that these cells may enter such areas through random motion and then become trapped. They may arise from the tissue of the part involved and accumulate

because of an increased rate of cell division. On the other hand, it is possible that these cells are chemotactically attracted to the inflammatory focus by substances or environmental conditions which have not yet been tested experimentally. The delayed appearance of the monocyte in the inflammatory area, in contrast to the prompt arrival of the polymorphonuclear, suggests also that time may be an important factor, and it is possible that observations carried on over a longer period, perhaps several days or a week, would reveal a latent attraction of the monocyte toward the bacteria.

SUMMARY

The chemotactic reactions of rat monocytes have been compared with those of polymorphonuclear leukocytes and lymphocytes. The responses of these cells to Staph. aureus, the tubercle bacillus and an aluminum silicate have been studied in tissue culture. It was found that whereas polymorphonuclears show positive chemotaxis to both Staph. aureus and the tubercle bacillus, monocytes and lymphocytes under similar conditions fail to exhibit chemotaxis. Toward aluminum silicate polymorphonuclears exhibit strong negative chemotaxis, while monocytes are weakly repelled and lymphocytes show no reaction.

It is concluded that the monocyte, lymphocyte and polymorphonuclear differ in their chemotactic behavior, at least in the rat. Possible explanations for the appearance of monocytes in inflammatory foci have been mentioned and the course of further investigation suggested.

INTRAVITAL STAINING OF MALIGNANT NEOPLASMS IN MAN BY EVANS BLUE

ALEXANDER BRUNSCHWIG, M.D.

ROBERT L. SCHMITZ, M.D.

AND

T. HOWARD CLARKE, M.D.

CHICAGO

The intravital staining of malignant neoplasms as a therapeutic measure has been studied in animals. A wide variety of dyes has been employed, and in general the results have been negative so far as retardation of tumor growth is concerned.[1]

While injections of intravital stains have undoubtedly from time to time been made in man as a therapeutic measure for cancer there are only two reports of systematic observations of such treatment in the literature. Roosen [2] and later Bernhardt [3] employed isamine blue for injections in cases of advanced malignant neoplastic disease. The latter author in a series of over 50 cases of various types made repeated injections of 5 to 20 cc. of a 0.5 to 0.8 per cent solution until twenty-four injections had been given. Generalized staining of the patients was observed, with increased concentrations of the dye in and about the neoplasms (stroma of the tumors) and about other inflammatory lesions, such as tuberculous foci. Other substances, such as a copper-silver preparation (kuprokollargol) and neoarsphenamine, were administered as adjuvants, and it was felt that definite palliative effects were observed in some instances.

Recently Duran-Reynals [4] observed that Evans blue (an isomer of trypan blue), among other electronegative blue azo dyes, when injected intravenously

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This work was conducted under a grant from the Cancer Research Institute of the Chicago Woman's Club.

From the Department of Surgery and the Division of Roentgenology of the Department of Medicine of the University of Chicago.

1. (*a*) Goldmann, E. E.: Neue Untersuchungen über die aüssere und innere Sekretion des gesunden und kranken Organisms, Tübingen, Verlag H. Lauppschen, 1912. (*b*) Weil, R.: J. Cancer Research **1**:95, 1916. (*c*) Marsh, M. C., and Simpson, B. T.: ibid. **11**:417, 1928. (*d*) Ludford, R. J.: Proc. Roy. Soc., London, s.B **104**:493, 1929.

2. Roosen, R.: Deutsche med. Wchnschr. **49** (pt. 1):577, 1923.

3. Bernhardt, H.: Ztschr. f. Krebsforsch. **27**:221, 1928.

4. Duran-Reynals, F.: Am. J. Cancer **35**:98, 1939.

into mice, rabbits and chickens bearing spontaneous and transplanted neoplasms produced general staining with quite marked selective localization in and about the neoplasms and their metastases. The tumor cells themselves did not absorb the dye; it accumulated in the fibroblasts and macrophages of the stroma.

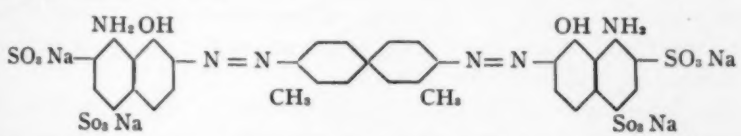
These observations were confirmed by us in rats bearing benzpyrene sarcoma of the subcutaneous tissues and in rabbits with benzpyrene carcinoma of the ears. Furthermore, evidence of the selectivity of the dye for malignant neoplastic foci as compared with benign neoplasms was afforded by the observation that in 3 rabbits bearing squamous cell carcinoma of the ears, the result of benzpyrene applications, and a variety of warts and benign sessile and pedunculated papillomas, there was, during the twenty-four hours following intravenous injection of 15 mg. of Evans blue, concentration of the dye in and about the malignant tumors but not in or about any of the benign lesions. In 2 rats bearing benzpyrene sarcoma of the subcutaneous tissues of the back there was also spontaneous adenofibroma of the breast; twenty-four hours after intravenous injection of 5 mg. of the dye there was concentration of it in and about the sarcoma but not in or about the benign mammary tumor. In 2 other rats bearing benign adenofibroma of the breast similar injections were made, and autopsy twenty-four hours later revealed in each case the presence of fibrosarcoma in the gastric wall, which was stained a dark turquoise blue, whereas the mammary tumors exhibited the light blue discoloration common to all the fibrous and adipose tissue in the body.

In all of the animal experiments mentioned the concentration of the dye in the liver and spleen was variable; the erythroblastic marrow was never grossly discolored; the mesenteric lymph nodes, especially those near the cecum, frequently showed concentration of the dye almost equal to that in the malignant tumors. The dye was regularly concentrated in the kidneys and perinephric fat. The intensity of the general staining of all mesoblastic tissues depended on the dose injected. A curious phenomenon, often noted, was the concentration of the dye in the fat and fibrous tissue beneath the sternum and in that of the abdominal wall bordering the diaphragm.

The described properties of Evans blue are of interest because this dye has been employed clinically in man for determinations of blood volume.[5]

Studies were undertaken to determine whether in man also the dye would localize selectively in the stroma of, and tissues about, malignant neoplasms.

The Evans blue, formerly known as T 1824, was purchased from the Eastman Kodak Company, Rochester, N. Y. Its structural formula as given by Gibson and Evans [5c] is:



Experience in over 300 patients [5c] with doses of 0.002 mg. per kilogram indicated no toxicity, and, when tested in rats, doses of 20 mg. per kilogram showed no

5. (*a*) Dawson, A. B.; Evans, H. M., and Whipple, G. H.: Am. J. Physiol. **51**:232, 1920. (*b*) Gregerson, M. I.; Gibson, J. J., and Stead, E. A.: ibid. **113**:54, 1935. (*c*) Gibson, J. G., and Evans, W. A.: J. Clin. Investigation **16**:301, 1937.

toxicity.[6] With the cooperation of Dr. Sherman Jennings, we observed that in newborn rats, with litter mates as controls, doses of 50 mg. per kilogram injected intraperitoneally were without toxic effects but that doses of 100 mg. per kilogram were lethal in a few instances and resulted regularly in retardation of growth and impairment of the general condition.

Solutions for intravenous injection in patients were made as follows: From 40 to 50 cc. of physiologic solution of sodium chloride was prepared and sterilized in the routine manner for intravenous use. Thirty to 100 mg. of the dye was weighed out and immediately added to the salt solution, which was well shaken to obtain complete solution of the dye, and the mixture placed in an oven kept at 56 C., for one to three hours. Intravenous injections were carried out rapidly, and in no instance caused immediate or delayed reactions. Where some of the solution escaped into the subcutaneous tissues, no inflammatory reactions were ever observed to develop. A few minutes after the injection the sclera appeared to be tinged blue. The next day the skin of the entire body appeared dusky, the hue varying with each patient and not related to age, cachexia or other condition. The skin of the hands, face (especially the forehead) and genitalia were more discolored than the other surfaces. This discoloration faded in several weeks, with return to a normal complexion.

The results of the injections are summarized in the table.

Summary of Results Observed on Intravenous Injection of Evans Blue in Patients with Malignant Neoplasms

Diagnosis	Patients Showing Selective Concentration of Dye in Neoplasms	Patients Showing No Concentration of Dye in Neoplasms
Carcinoma of skin	3	2
Carcinoma of lip	1	..
Carcinoma of larynx	..	1
Carcinoma of breast	7	1
Carcinoma of pancreas	2	..
Carcinoma of stomach	3	..
Carcinoma of liver	..	1
Carcinoma of gallbladder	..	1
Carcinoma of colon	2	2
Carcinomatosis (metastases in skull)	1	..
Osteosarcoma	1	..
Lymphoblastoma	..	2
Total	20	10

In the table, selective blue discolorations in and about the neoplasms are recorded as positive results, in contrast to the very slight to slight discoloration of the normal tissues of the rest of the body with which comparisons in each case are made. These results were recorded twenty-four hours after injection and on inspection of the lesions if they were superficial or on direct visualization at laparotomy, or at necropsy performed several days after injection.

As shown by the table, 20 of the 30 patients exhibited selective concentration of the dye in and about the neoplastic foci. Failure of selective concentration of the dye in the malignant neoplasms in 10 patients may have been due to a variety of causes and not necessarily to primary

6. Gibson, J. G., and Gregersen, M. I.: Am. J. Physiol. **113**:50, 1935.

impermeability of the vascular beds of these tumors. For example, 1 of the 2 skin cancers failing to localize the dye had been treated moderately with roentgen radiation, which resulted in partial sclerosis of the lesion. The carcinoma of the larynx had also been irradiated heavily (4,000 roentgens) several months previously. The carcinoma of the liver was so extensive that localization of the dye might have occurred and not been appreciated because of dilution of the dye in the large tumor masses.

In several instances two or more injections were made at daily intervals before selective localization became manifest. In certain instances in which there were widespread metastases only some of the latter localized the dye. It is possible that in some cases in which selective concentrations were not observed injections of greater doses than were employed would have resulted in the demonstration of the phenomenon. Necrotic portions of the neoplasms never exhibited the bluish discoloration.

There is no correlation between the general histologic type of a neoplasm and its ability to localize the dye. In a number of the cases benign neoplasms, such as cavernous hemangioma of the skin and nevi, were also present, but these failed to localize the dye. In 2 instances of cerebral astrocytoma there was no localization of the dye in the tumor. One patient presented infiltrating multiple nodular masses in the abdominal wall, which had recurred repeatedly after excisions over a period of thirteen years. Microscopic examination of the tissue afforded a picture strongly suggestive of fibrosarcoma, but to date no metastases have developed. Injection of Evans blue did not result in localization of the dye in and about the last "crop" of these recurrences.

While frozen sections for microscopic study were not made in the majority of cases, the fact that the dye was observed in the non-neoplastic tissue immediately about the focus of tumor cells, as well as throughout the viable portions of the latter, would indicate that it had localized in the fibroblasts and macrophages in these areas and not in the tumor cells themselves, as has been adequately demonstrated to be the manner of localization for this and other similar dyes injected into tumor-bearing animals.[7] An interesting report has recently been published on the necropsy of a patient treated a number of years ago with injections of isamine blue by Bernhardt. In this case granules of the dye were still present in the tissues, including the epithelial cells.[8]

COMMENT

As observed in patients coming to necropsy, the dye employed in this study did not, in addition, selectively concentrate in the liver, spleen,

7. Ludford,[1d] Duran-Reynals.[4]

8. Hamperl, H.: Ztschr. f. Krebsforsch. **49**:348, 1939.

bone marrow and lymph nodes, the tissues which normally include so large a portion of the actively phagocytic cells of the reticuloendothelial system in direct contact with the blood stream. Duran-Reynals called attention to this in his studies on tumor-bearing mice. When he injected india ink into these animals, it did not localize in or about the neoplasms. These observations were confirmed by us in 2 rabbits with squamous cell carcinoma of the ear and 3 rats with benzpyrene sarcoma of the subcutaneous tissues of the back.

It would thus appear that the selective localization of Evans blue in and about neoplastic foci in man is, as in the case of animals, dependent on some factor associated with a local increase in capillary permeability to the dye. Indeed, this would explain why necrotic portions of tumors and certain sclerotic or relatively avascular portions of them were not observed to localize the dye selectively. Furthermore, a variation in the physiologic state of the capillary beds in various neoplastic foci may also account for the fact that uniform staining of all tumor foci was not observed in any patient.

Additional evidence of the importance of the permeability of the tumor's capillary bed in the localization of the dye is furnished by 2 patients in our series. In one, who presented a large chronic ulcerating lesion of the thigh, only those portions of the lesion which biopsies revealed to be carcinomatous were stained blue. The discoloration was due essentially to the dye in the exudate over the neoplastic tissue, for when this was wiped away there remained visible in the lesions only small scattered blue spots; the exudate and the granulating surface of the non-neoplastic portion of the lesion (as shown by biopsy) were not stained blue. The other patient had a flat ulcerating carcinoma of the lower lip, and here, too, the bluish color that appeared over the surface twenty-four hours after injection of the dye could for the most part be removed by wiping away the dried exudate over the lesion. The exudate that developed about radium needles that had been inserted through normal tissue at the margins of the lesion did not appear blue during the first forty-eight hours following injection of the dye.

It has been repeatedly demonstrated that trypan blue is rapidly localized early in the evolution of acute inflammatory lesions of pyogenic origin or lesions produced by strong chemical irritants. Fox [9] called attention to the fact that older lesions (of five days' duration) of this type do not as readily localize the dye or may not localize it at all. We have observed that Evans blue injected into 2 patients with large chronic granulating bed sores was not localized in the lesions until after three or four days and then not as intensely as in malignant neoplasms; and in a third patient it failed to localize in a sinus with a chronic discharge from a deep pyogenic abscess. It thus appears that the localization of

9. Fox, J. P.: J. Immunol. **31**:293, 1936.

Evans blue in and about malignant neoplastic foci could hardly be accounted for simply on the basis of a granulation tissue type of inflammation occurring in and about such foci, which are of at least several weeks' duration.

Attempts to Increase the Permeability of the Tumor's Vascular Bed to the Dye by Means of Histamine.—In 3 patients who presented multiple cutaneous recurrences of cancer of the breast, 50 mg. of Evans blue was injected intravenously, and thirty minutes later histamine wheals were produced in the uninvolved skin and in the skin to include one or more neoplastic nodules. Repeated observations over a period of twenty-four hours showed that there was no increase in the rapidity or intensity of the localization of the dye in the cutaneous tumor nodules which had received injections of histamine as compared with the site of histamine wheal formation in the normal skin. In other words, histamine brought about maximum permeability in the capillaries whether these happened to be in normal tissue or in the stroma of malignant neoplasms.

SUMMARY

Previous observations that Evans blue (T1824) localizes selectively in and about malignant tumors in animals are confirmed.

Observations are recorded showing that such selective localization of the dye occurred in a majority of a series of human subjects presenting a variety of malignant neoplasms.

In some of the treated animal and human subjects benign neoplasms were also present, and these, in contrast to the malignant growths, did not show localization of the dye.

Case Reports

ACCESSORY PANCREAS IN THE WALL OF THE GALLBLADDER

ABRAHAM S. JACOBSON, M.D., NEW YORK

Since Klob[1] first described aberrant pancreatic tissue there have been many reports of this anomaly. The most frequent locations for the aberrant tissue are the stomach and duodenum; following these, the jejunum is a close third, while the ileum and peritoneal lining are a poor fourth and fifth. The aberrant tissue is rarely located in the gallbladder or the spleen.

The first reported case of pancreatic tissue in the human gallbladder was that of von Hedry.[2] After a cholecystectomy on a 40 year old woman, the gallbladder, which showed the characteristics of chronic cholecystitis, was found to contain a subserous structure about the size of a bean. This structure grossly and microscopically had the appearance of pancreatic tissue. Normal acini and ducts and two typical islands of Langerhans were seen. Von Hedry did not state the proportion of ducts to acini in the tissue. No clinical symptoms could be attributed to the presence of the aberrant tissue.

Two years before von Hedry, Mann[3] reported a case of accessory pancreas in the gallbladder of a dog. The aberrant tissue was subserosal, measured 1.5 by 1 cm., and varied from 2 to 4 mm. in thickness. It was normal pancreatic tissue, with a well developed duct system, but the acini seemed to be rather small, and only a few definite, very small islands were found. The gallbladder wall underlying the aberrant tissue did not appear different from any other portion except that there were three pinpoint openings which under the dissecting microscope appeared to be openings of ducts. In the histologic sections it was impossible to follow any of the ducts through to an orifice in the gallbladder, although ducts were present near the site of the apparent openings. Mann's comment is of interest: "The wide distribution of accessory pancreases along the stomach and small intestine, and the fact that the ventral pancreas normally communicates with the bile duct, lead one to anticipate the possibility of finding occasionally an aberrant gland opening into the gallbladder, but thus far there seems to be no case on record of such an occurrence."

In 1928 Cogniaux[4] reported 8 cases of aberrant pancreas. Among these was a case in which a small amount of aberrant pancreatic tissue was found under the mucosa of the gallbladder after cholecystectomy on a 39 year old woman. The tissue consisted of three or four lobules without any excretory ducts or islands of Langerhans. The acini appeared normal.

From the Department of Pathology, Beth Israel Hospital.

1. Klob, J.: Ztschr. d. k.-k. Gesellsch. d. Aerzte z. Wien **15**:732, 1859.

2. von Hedry, N.: Beitr. z. klin. Chir. **132**:570, 1924.

3. Mann, F. C.: Anat. Rec. **23**:351, 1922.

4. Cogniaux, P.: Arch. franco-belges de chir. **31**:307, 1928.

One other reference to aberrant pancreatic tissue in the wall of the gallbladder appears in Gruber's text.[5] "Richard Simon (Pankreolithiasis mit akzessorischem Pankreas am Pylorus, Med. Inaug. Dissert. Heidelberg, 1925) found a bean-sized node in the neck of an extirpated gallbladder which histologically was found to be accessory pancreas with ducts but without definite islands." Further details are not available, since this dissertation could not be obtained for personal reading.

Pancreatic acini and ducts in the lamina propria of the gallbladder wall; hematoxylin-eosin stain.

Microscopic examination of a gallbladder at the pathologic laboratory of the Beth Israel Hospital has added another case to the roster.

A 19 year old woman with a three year history of signs and symptoms diagnosed as those of cholecystitis and cholelithiasis was admitted Oct. 4, 1939 with an attack of colicky pain in the epigastrium and pains in the shoulders and back. Cholecystectomy was done October 5. The patient made an uneventful recovery.

5. Gruber, in Henke, F., and Lubarsch, O.: Handbuch der pathologischen Anatomie, Berlin, Julius Springer, 1929, vol. 5, pt. 2, p. 261.

Gross examination showed the gallbladder to be 11.5 cm. long. It was hyperemic and tightly filled with more than one hundred small green faceted cholesterol-pigment stones. The mucosa was light reddish brown and appeared intact.

Microscopic examination showed the mucosa to be well preserved. In the muscle coat, notably in its outer layers, a considerable degree of chronic inflammation was seen. At one point in the lamina propria an epithelial structure was situated, which differed from the normal mucosa. Further examination showed it to consist of typical pancreatic acini and ducts approximately in the proportion of 1:1. No islands of Langerhans could be seen. The aberrant tissue measured about 0.15 by 0.15 mm. Further sections of the original block and also additional blocks of the gallbladder tissue were examined. The aberrant tissue could be traced for only about 60 microns and could not be picked up again in the additional blocks. It is possible that in preparing the original block the major part of the aberrant tissue was discarded and only the tail end retained.

This case does not throw any light on the various theories as to the origin of accessory pancreatic tissue. A review of these theories can be found in a paper by Branch and Gross.[6]

SUMMARY

A fourth case of aberrant pancreatic tissue in the gallbladder of man is added to the 3 cases reported in the literature. (While this paper was in press another case of an accessory pancreas in the gallbladder was reported.[7] The pancreatic tissue [10 by 5 mm.] was found in the neck of the extirpated gallbladder of a 34 year old woman. It contained islands. At several points the pancreatic tissue was continuous with the glandular structures in the neck of the gallbladder.)

6. Branch, C. D., and Gross, R. E.: Arch. Surg. **31**:200, 1935.
7. Thorsness, E. T.: Anat. Rec. **77**:319, 1940.

SYPHILITIC (GUMMATOUS) PULMONARY ARTERITIS WITH RUPTURE INTO THE BRONCHIAL TREE

A. J. Segal, M.D., Montreal, Canada

Two types of syphilitic disease of the pulmonary artery are recognized.[1] One is a productive cicatricial lesion similar to that found in syphilitic aortitis. The other is a gummatous form in which miliary gummas, well circumscribed, are found in the adventitia, media or intima of the artery with necrosis and partial destruction of the affected layer. The productive cicatricial type is frequently associated with aneurysm formation and may be accompanied by organized thrombi. The gummatous type is not associated with aneurysm but with focal necrosis. Such foci of necrosis are potential points of rupture into one of the serous sacs, into the lung or into an adjacent bronchus. Peck [1a] maintained that the gummatous type is the more common, basing his statement on the cases he reported and reviewed. However, the incidence of each type is approximately equal in the cases reported up to the present time.

Reviews of the literature up to 1909 by Henschen [2] and Posselt [3] indicate that early observers suspected a syphilitic basis for arteriosclerotic changes in the pulmonary artery. In most of these cases, however, the pathologic description has been inadequate, so that the true nature of the underlying process is obscure.

In a review of the literature up to 1927 Peck [1a] cited 12 cases of syphilis of the pulmonary artery. In 3 of these [4] the disease was of the productive cicatricial type and in the remainder it was of the gummatous variety. Four additional cases of the latter type have been subsequently reported.[5] Karsner [1b] reviewed the lesions of the productive cicatricial type and cited 8 additional cases. Seven other cases of this variety are considered acceptable.[6]

From the Department of Laboratories, Jewish General Hospital.

1. (*a*) Peck, S. M.: Arch. Path. **4**:365, 1927. (*b*) Karsner, H. T.: Arch. Int. Med. **51**:367, 1933.

2. Henschen, S. E.: Samml. klin. Vortr., 1906, no. 422-423 (Inn. Med. no. 126-127), p. 595.

3. Posselt, A.: Ergebn. d. allg. Path. u. path. Anat. **13**:298, 1909.

4. (*a*) Barth, H.: Frankfurt. Ztschr. f. Path. **5**:138, 1910. (*b*) Warthin, A. S.: Am. J. Syph. **1**:693, 1917. (*c*) Henschen.[2]

5. Kux, E.: Ztschr. f. Kreislaufforsch. **24**:1, 1924. Clarke, R. C.; Coombs, C. F.; Hadfield, G., and Todd, A. T.: Quart. J. Med. **21**:51, 1927. Wassiljeff, A. A., and Argun, A. M.: Centralbl. f. allg. Path. u. path. Anat. **47**:327, 1929. Poinso, R.: Gaz. d. hôp. **100**:477, 1927.

6. Brenner, O.: Arch. Int. Med. **56**:1189, 1935. Wahl, H. R., and Gard, R. L.: Surg., Gynec. & Obst. **52**:1129, 1931. Salzer, G.: Centralbl. f. allg. Path. u. path. Anat. **41**:100, 1928. D'Aunoy, R., and von Haam, E.: J. Path. & Bact. **38**: 39, 1934. Laubry, C., and Thomas, M.: Bull. et mém. Soc. méd. d. hôp. de Paris **51**:9 1927. Reimann, S. P.: Proc. Path. Soc. Philadelphia **22**:42, 1920. Pissot, P. M.: Contribution à l'étude de l'anévrysme de l'artère pulmonaire, Thesis, Paris, no. 379, 1920.

In summarizing, it appears that 13 cases of gummatous type and 18 cases of the productive cicatricial type have been reported which are acceptable as proved cases of syphilis of the pulmonary artery. In only a single case [4b] have spirochetes been demonstrated within the vessel wall.

REPORT OF A CASE

A 50 year old white fireman was first admitted to this hospital Dec. 26, 1938. His chief complaints were pain in the chest, dyspnea, productive cough and foul breath, of one month's duration. In the five days preceding admission he had had several hemoptyses of undetermined quantity.

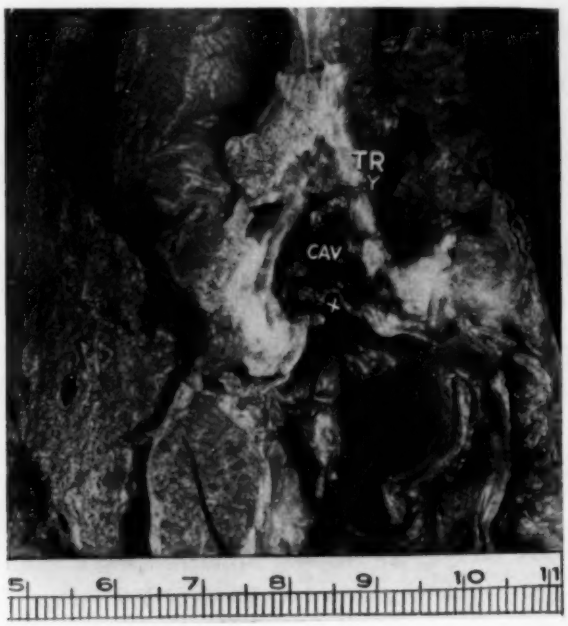


Fig. 1.—Cross section through the cavity in a fixed specimen. *TR*, is the tracheal wall; *Y*, the point of rupture into the trachea; *CAV*, the blood filled cavity; *X*, the point of communication with the right main pulmonary artery below.

From 1914 to 1934 he had received occasional antisyphilitic therapy. However, the Wassermann reaction of the blood remained strongly positive. Since 1928 he had had three attacks of pain in the chest and dyspnea with hemoptysis of undetermined quantity, similar to the recent episodes.

Clinical and roentgenologic examination led to a diagnosis of pneumonia involving the upper lobe of the right lung and chronic bronchiectasis. In view of the previous history and the strongly positive reaction of the blood in the Wassermann, Kahn and Kline tests an additional diagnosis of tertiary syphilis was made. He was discharged, improved, after ten days.

Six days later the patient was readmitted in a critical condition because of sudden repeated massive hemoptysis. On admission he had two further hemoptyses, of 500 to 600 cc. each. The blood pressure, which had been 60 systolic and 50 diastolic,

was then unobtainable. The radial pulse was not palpable. In spite of repeated transfusions of large amounts of blood and general supportive therapy, he died twenty-seven hours later, following a final massive hemoptysis.

At autopsy, made nine hours after death by Dr. M. A. Simon, the body was that of an extremely well developed and well nourished man. There was moderate cardiac hypertrophy and dilatation with slight fibrosis of the myocardium of the left ventricle. The aorta showed longitudinal wrinkling of the intimal surface of the arch. The trachea contained a large amount of brownish-red semifluid material. On the right lateral aspect of the trachea, at the level of the bifurcation, there was a circular defect in the wall, measuring 1 mm. in diameter, which communicated

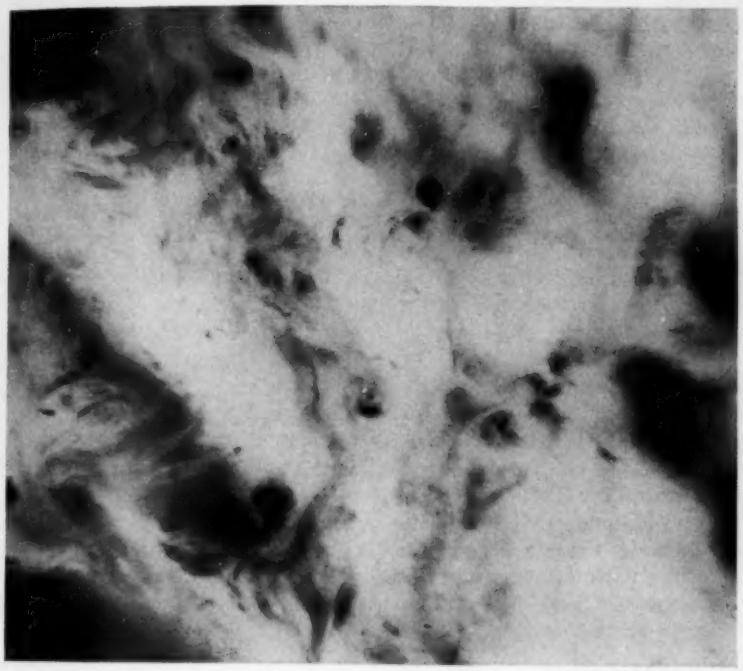


Fig. 2.—Oil immersion photomicrograph showing tightly coiled spirochetes (S. pallida). Heitzman stain.

with a cavity lying paratracheally on the right (fig. 1). This cavity measured 3 by 2 cm. and contained a large amount of friable clotted blood. The inner surface was ragged and irregular. This cavity communicated inferiorly, through a circular defect, with the lumen of the right main pulmonary artery. The defect in the arterial wall measured 3 by 2 mm. The edges were irregular, mottled green, red and black, and were inverted for a short distance into the large cavity. The lateral wall of the cavity was made up of mediastinal fibroadipose tissue and black mottled firm tissue, possibly lymph node. The entire cavity was walled off by the trachea and the mediastinal fibroadipose tissue. The fistulous communication extended between the right main pulmonary artery and the trachea. The bronchial tree of each lung was filled with blood, and complete dissection failed to confirm the clinical impression of bronchiectasis.

Microscopic examination of the aorta showed typical syphilitic aortitis. Sections were taken through the right main pulmonary artery to include it, the wall of the cavity and the trachea. At the edge of the defect elastica stains showed disruption and fragmentation of the elastic fibers. There was no evidence of pulmonary arteriosclerosis. In the adventitia of the artery, small hyalinized lamellated nodules were found at a point somewhat removed from the defect. Sections stained by the Heitzman method [7] showed slender spirochetes resembling Spirochaeta pallida in the media of the pulmonary artery and at some distance removed from the edge of the defect (fig. 2). The arterial wall near the defect was diffusely infiltrated by lymphocytes, plasma cells and rare polymorphonuclear leukocytes. The wall of the cavity showed mild deposition of anthracotic pigment and large acellular hyalinized lamellated nodules, in which there was a small amount of anthracosis. Several atypical large multinucleated giant cells were seen, but no typical areas of caseation necrosis were present. Sections stained by the Ziehl-Neelsen method failed to show acid-fast bacilli. No lymph node structures could be identified in the wall of the cavity.

Other observations were noncontributory.

COMMENT

The genesis of this lesion remains somewhat obscure in that the initial focus of syphilitic infection cannot be determined with accuracy. Because of the secondary changes associated with hemorrhage and nonspecific infection in the tissue between the pulmonary artery and the trachea, the possibility that one is dealing with gumma in an extravascular position and with subsequent extension into the pulmonary artery cannot be excluded. However, the finding of spirochetes resembling S. pallida well within the media and at some distance from the inner surface of the cavity suggests that the lesion was primary in the arterial wall.

If one assumes that the tissue replaced by the cavity was originally a lymph node, there are other factors which might have led to its disintegration, with subsequent extension into the pulmonary artery. These are anthracosis and tuberculosis. These may be excluded for the following reasons:

The degree of anthracosis present was minimal and not sufficient to have produced this cavity, nor was there evidence of excessive anthracosis elsewhere in the lung.

Some features of the microscopic lesion suggest those seen in tuberculous infection, but sections stained by the Ziehl-Neelsen method failed to show acid-fast bacilli.

The fact that the hemorrhagic cavity communicated with the trachea and hence with the oral cavity raises the question as to whether the spirochetes seen and demonstrated in this lesion are really S. pallida. Morphologically, the spirochetes seen are longer, thinner and more tightly coiled than are the oral spirochetes. The latter are short, thick and loosely coiled. Furthermore, the fact that the spirochetes in this case do not lie within the cavity but are found deeply situated in the

7. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938.

media and adventitia of the pulmonary artery at some distance from the inner surface of the cavity precludes these spirochetes as secondary invaders.

The patient was known to be syphilitic and untreated for many years. He had strongly positive serologic evidence of syphilis, and he had syphilitic aortitis. The pulmonary arterial tree was conspicuously free of sclerotic changes, and the probability of focal arteriosclerosis is remote.

The history of repeated small hemoptyses occurring several weeks before the massive fatal one and the history of three small hemoptyses during the previous ten years suggest that the syphilitic process was progressive and that a minute communication between the pulmonary artery and the trachea had already been established. The exact duration of the syphilitic lesion in the pulmonary artery cannot, however, be determined on the basis of the material at hand. Obviously it was a late tertiary manifestation of syphilis and, as such, was slowly progressive, and must be regarded as of considerable duration. There is no proof that secondary infection hastened the fatal outcome.

SUMMARY

A case of syphilitic gummatous pulmonary arteritis with rupture and massive hemorrhage into the bronchial tree has been presented.

INTRATHORACIC PHEOCHROMOCYTOMA

BENJAMIN PHILIPS, M.D., NEW YORK

Tumor of the adult chromaffin cells is rare. In 1937 Wells and Boman,[1] in an excellent review, reported that 82 cases had been described in the literature and recorded 1 case of their own. There have since been several scattered case reports.[2] In the vast majority of the cases the tumor has arisen from the chromaffin cells of the adrenal medulla. Disregarding tumor of the carotid body, the status of which in the chromaffin system is disputed, I have been able to find only 11 cases reported in which pheochromocytoma (chromaffinoma, paraganglioma) was found unassociated with the adrenal gland. In 9 cases [3] the tumor arose in Zuckerkandl's organ, on the ventral aspect of the aorta just above its bifurcation; in one [4] it was found in a retroperitoneal ganglion near the origin of the superior mesenteric artery; in another [5] the tumor was found in the right pleural cavity paravertebrally at the level of the sixth intercostal space. I have recently encountered a case in which a pheochromocytoma was found at the apex of the left pleural cavity paravertebrally at the level of the first intercostal space.

REPORT OF CASE

A 39 year old white man related the onset of his illness to incision of a peritonsillar abscess at the age of 29, following which he began to experience episodes of cardiac palpitation and throbbing in the head. He also noted dyspnea, polyuria and nocturia. At this time he was told that he had high blood pressure. These episodes occurred repeatedly for three years, and after a short period of hospitalization he was told that he had chronic diffuse glomerulonephritis. One year later, at the age of 33, he suddenly experienced left hemiplegia, with subsequent gradual improvement.

From the Laboratory Division of the Montefiore Hospital for Chronic Diseases.

1. Wells, A. H., and Boman, P. G.: J. A. M. A. **109**:1176, 1937.

2. (*a*) MacKenzie, D. W., and McEachern, D.: J. Urol. **40**:467, 1938. (*b*) Palmer, R. S., and Castleman, B.: New England J. Med. **219**:793, 1938. (*c*) Brunschwig, A.; Humphreys, E., and Roome, N.: Surgery **4**:361, 1938. (*d*) Holst, E. J.: Acta med. Scandinav. **94**:510, 1938. (*e*) Howard, J. E., and Barker, W. H.: Bull. Johns Hopkins Hosp. **61**:371, 1937.

3. (*a*) Gellerstedt, N., and Thyresson, N.: Upsala läkaref. förh. **44**:303, 1939. (*b*) Reichardt, R.: Endokrinologie **14**:180, 1934. (*c*) Cragg, R. W.: Arch. Path. **18**:635, 1934. (*d*) Bauer, J., and Leriche, R.: Presse méd. **42**:1385, 1934. (*e*) Merkulow, G. A.: Centralbl. f. allg. Path. u. path. Anat. **59**:274, 1934. (*f*) Nordmann, M., and Lebküchner, E.: Virchows Arch. f. path. Anat. **280**:152, 1931. (*g*) Handschin, E.: Beitr. z. path. Anat. u. z. allg. Path. **79**:728, 1928. (*h*) Hausmann, M., and Getzowa, S.: Schweiz. med. Wchnschr. **52**:889 and 911, 1922. (*i*) Stangl, E.: Verhandl. d. deutsch. path. Gesellsch. **5**:250, 1902.

4. Mayo, C. H.: J. A. M. A. **89**:1047, 1927.

5. Miller, J. W.: Centralbl. f. allg. Path. u. path. Anat. **35**:85, 1924.

In October 1933, shortly after the aforementioned episode, he was admitted to Montefiore Hospital for study. He was a well developed and well nourished plethoric person, not appearing acutely ill, with residual left hemiparesis. The retinal arterioles were pale, shiny and markedly constricted; and the ocular disks were slightly edematous. The heart was enlarged, with the point of maximum impulse in the fifth intercostal space, slightly outside the midclavicular line. There was a soft systolic murmur over the entire precordium, with accentuation of the second sound over the apex and aortic area. The blood pressure in the right arm was 200 systolic and 120 diastolic, and that in the left, 170 systolic and 126 diastolic. The urine had a maximum specific gravity of 1.023, a trace of albumin, a few white blood cells and occasional red blood cells, with no casts. Fluoroscopic and roentgenographic examination of the chest (fig. 1) showed a soft oval mass lying in the left apex, which fluoroscopic examination revealed lay posteriorly,

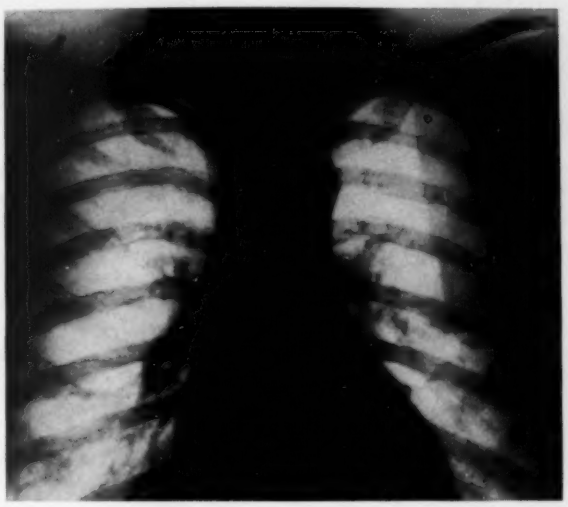


Fig. 1.—Roentgenogram of the chest on the patient's first admission to the hospital. Note the oval mass in the left apex.

did not move on swallowing and did not compress the trachea. The heart was vertical and showed a moderate rounding and elongation of the aorta. An electrocardiogram revealed normal sinus rhythm with left axis deviation.

During two uneventful months in the hospital the patient's blood pressure varied from 170 systolic and 120 diastolic to 220 systolic and 120 diastolic. He then attended the cardiac clinic for five years, during which time he complained of occasional cardiac palpitations. His blood pressure varied from 160 systolic and 124 diastolic to 204 systolic and 140 diastolic. Here it was noted for the first time that he had Horner's syndrome on the left, with narrowing of the palpebral fissure, enophthalmos, miosis and anhidrosis of the left side of his face. In June 1939 he was admitted to another hospital with acute coronary occlusion, following which congestive heart failure appeared, and he was transferred to Montefiore Hospital.

At this time the pertinent physical findings were: residual hemiparesis and Horner's syndrome on the left; thin, slightly tortuous retinal arterioles with distended veins and slight arteriovenous nicking, and a small area of exudate

in the right fundus; fulness of the left supraclavicular fossa, with slight tenderness on palpation; bilateral pleural effusion; enlarged heart with the cardiac impulse in the sixth intercostal space just within the anterior axillary line, fair heart sounds, accentuated second aortic sound, no thrills or murmurs and regular sinus rhythm. The urine reached a specific gravity of 1.015 and contained occasional red and white blood cells but no albumin. The blood urea nitrogen was 25.8 mg. per hundred cubic centimeters. An electrocardiogram showed left axis deviation, low voltage and deep Q waves in leads 2, 3 and 4. The man died nine days after admission, with increasing stupor and Cheyne-Stokes respiration.

Post mortem the pertinent observations were as follows:

At the apex of the left pleural cavity there was a firm mass about the size of a plum, not attached to the lung. It was easily shelled out and removed. Unfortunately, because of the limitations of an abdominal incision, the exact anatomic relationships of this mass to adjacent structures could not be determined. On gross section the tumor consisted of firm white areas alternating irregularly with soft hemorrhagic purple areas. Microscopically (fig. 2), it consisted of nests of large polyhedral cells in a richly vascularized fibrous tissue stroma. The cell outlines were indistinct; the cytoplasm was granular; the nuclei were large and vesicular and usually contained one large nucleolus. In many areas of sections fixed in Orth's solution, which contains potassium dichromate, the cytoplasmic granules were pigmented golden yellow. In sections fixed in solution of formaldehyde U. S. P. these pigmented granules were absent. There were small scattered areas of hemorrhage and necrosis.

The adrenals together weighed 20 Gm. The cortex was distinct and contained a moderate amount of lipoid. Microscopically, there was shrinking of the glomerular zone with congestion of the reticular zone. Normal medullary tissue was present.

The heart was greatly enlarged weighing 700 Gm. There was marked hypertrophy of both ventricles, the left ventricle measuring 20 mm. in thickness, and the right 7 mm., with bulging of the interventricular septum into the right ventricle. A well healed large myocardial infarct involved the posterior wall, apex and apical portion of the anterior wall of the left ventricle. The remainder of the myocardium was red-brown and firm. There was marked atherosclerosis of the coronary arteries, with complete, well organized occlusions in the right coronary artery immediately distal to its origin and in the anterior descending branch of the left coronary artery about 3 cm. from its origin. All four valves were normal. There was a moderate amount of atherosclerosis of the aorta, with some ulceration and calcification of the plaques in the arch.

The kidneys each weighed 200 Gm. and measured 11 by 6 by 3 cm. Both capsules stripped with ease, revealing a dark red smooth surface. Both kidneys were soft and flabby and cut with ease. The cut surface was uniformly dark red, with almost complete obliteration of the parenchymal markings. The calices, pelves, ureters and bladder were normal. Microscopically, there was marked congestion with widespread arteriolosclerosis. The glomeruli varied in structure; some were completely hyalinized, others were small and shrunken, with thickening of Bowman's capsule, and still others were relatively normal.

COMMENT

According to Kohn,[6] who first described the chromaffin system, in 1903, chromaffin cells can be found along the entire length of the autonomic nervous system, occurring as nests in the autonomic gan-

6. Kohn, A.: Arch. f. mikr. Anat. **62**:263, 1903.

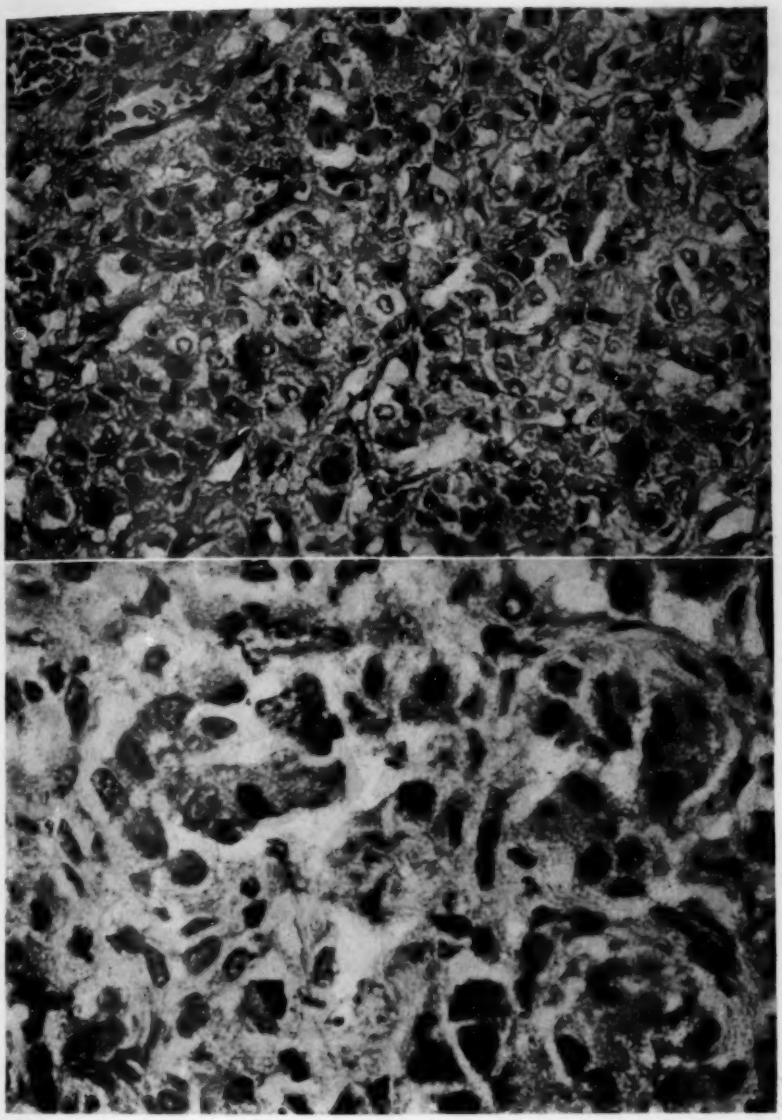


Fig. 2.—Upper: Low power photomicrograph of the tumor. The growth is composed of nests of large polyhedral cells in a vascular fibrous tissue stroma. Lower: High power photomicrograph of the tumor. Note the large cytoplasmic granules, which appear golden yellow in sections fixed in Orth's solution.

glions, where they are known as paraganglions, or independently as chromaffin bodies. The latter include the adrenal medulla and Zuckerkandl's organ, which is situated on the ventrolateral aspect of the abdominal portion of the aorta immediately above its bifurcation. One may expect, therefore, tumors derived from chromaffin tissue to be found in the paraganglions or in the independent chromaffin bodies.

In this case we believe that the pheochromocytoma arose from the paraganglion associated with the first left thoracic ganglion. First, it was seen roentgenologically at the apex of the left pleural cavity posteriorly. Second, during the last five years of observation of the patient Horner's syndrome was noted on the left, i. e., narrowing of the left palpebral fissure, enophthalmos, miosis and anhidrosis. This was apparently caused by involvement of the efferent sympathetic fibers as they pass through the first thoracic ganglion from the spinal cord on their way to the cervical sympathetic chain. In this regard this tumor suggests the "superior pulmonary sulcus" syndrome first described by Pancoast[7] in 1924.

Of great interest in this case was the occurrence of severe cardiovascular and renal disease in a comparatively young person. The frequent incidence of vascular disease with cardiac hypertrophy, arteriolar changes in the retina and evidences of renal damage in patients with pheochromocytoma has been noted by several workers[8] and has caused much speculation. A notable case is that of a 14 year old girl, reported by Kremer,[9] in which, associated with bilateral pheochromocytoma of the adrenal medulla, there were found at autopsy enlargement of the heart with atherosclerosis of the coronary arteries and aorta and arteriolar thrombosis of the kidneys. In several instances of pheochromocytoma large amounts of epinephrine have been recovered on chemical analyses and biologic tests of the tumor tissue.[10] This has led some investigators to the conclusion that there is a causal relationship between the hypersecretion of epinephrine by secretory tumor tissue, the paroxysmal episodes of hypertension which occur in many cases and the profound anatomic alterations which occur in the cardiovascular and renal systems in others. Cure of the syndrome of paroxysmal hypertension by surgical removal of a pheochromocytoma was first achieved by C. H. Mayo,[4] in 1927, and has since been successfully repeated in about 15 cases.[2a] This proves definitely an etiologic relationship of the tumor to the syndrome of paroxysmal hypertension. However, it is more difficult to prove the tumor a cause of cardiac hypertrophy and arteriolosclerosis, for numerous cases have been reported in which the pheochromocytoma was an incidental finding post mortem, with no evidence

7. Pancoast, H. K.: J. A. M. A. **83**:1407, 1924.

8. Volhard, F.: Die doppelseitigen hämatogenen Nierenerkrankungen, in von Bergmann, G., and Staehelin, R.: Handbuch der inneran Medizin, ed. 2, Berlin, Julius Springer, 1931, vol. 6, pt. 2, p. 1742. Palmer and Castleman.[2b] Bauer and Leriche.[3d]

9. Kremer, D. N.: Arch. Int. Med. **57**:999, 1936.

10. (*a*) Fischer, H.: Ztschr. f. klin. Med. **134**:184, 1938. (*b*) Beer, E.; King, F. H., and Prinzmetal, M.: Ann. Surg. **106**:85, 1937. (*c*) Belt, A. E., and Powell, T. O.: Surg., Gynec. & Obst. **59**:9, 1934.

of vascular disease.[11] This may be explained, however, either by absence of physiologic activity of the tumor tissue in these cases or by the fact that sufficient time had not elapsed since the onset of the tumor's activity to cause permanent organic vascular changes. In the case presented, since the tumor had been present for at least as long as six years, and since there was no evidence of chronic renal inflammatory disease post mortem, it may be inferred that the continued functional activity of the pheochromocytoma secreting a pressor substance into the blood stream was responsible for the persistent severe degree of hypertension, the marked cardiac hypertrophy and the widespread arteriolosclerosis in a patient who was only in the fourth decade of life.

SUMMARY

A case of pheochromocytoma is reported. The tumor was situated at the apex of the left pleural cavity, suggesting a "superior pulmonary sulcus" syndrome; it was associated with marked cardiovascular and renal disease in a patient of 39 years.

11. Labbé, M.; Tinel, J., and Doumer: Bull. et mém. Soc. méd. d. hôp. de Paris **46**:982, 1922. Wells and Boman.[1] Belt and Powell.[10c]

Laboratory Methods and Technical Notes

LOW TEMPERATURE PRESERVATION OF GROSS SPECIMENS

J. Howard Ferguson, M.D., Syracuse, N. Y.

The method to be described has been found satisfactory for the preservation of fresh gross material for a considerable period. The preserved material has been used both for the teaching of gross pathology to medical students and for clinical pathologic conferences.

The method requires a low temperature refrigerator for best results. The refrigerator room which my co-workers and I use for this purpose can be maintained at a temperature of 5 F. (—15 C.). We have also used the freezing compartment of a commercial electric refrigerator and have found that the minimum temperature obtained was 18 F. (—8 C.). This has been found to be satisfactory for small sections of clean organs but not entirely so for larger specimens or grossly infected material. More rapid freezing at lower temperatures appears to produce the best preservation.

Material to be preserved may be prepared by one of two methods. For the preservation of the cut surface appearance of such organs as liver, spleen and kidneys, a perfectly flat section of the fresh tissue is made, cleaned of blood or secretion and placed face downward on a glass plate slightly larger than the specimen. It is necessary to have perfect contact between the specimen and the glass, as frosting will occur on the glass in any air space that remains. The specimen is then placed glass down on a tray or shelf in the refrigerator. The section of tissue should be thick enough so that the contact will be maintained by the weight of the specimen. When very low temperatures (5 F.) are used, most specimens will freeze completely solid within a short time. There is considerable dehydration with shrinkage of the exposed tissue after a period of time, but the cut surface in contact with the glass plate remains fresh in appearance. When removed from the refrigerator the tissue will remain frozen and attached to the plate for a period of time dependent on the original temperature used. We have found the time always sufficient for demonstration purposes. When the specimen is brought into a warm room, there is a tendency for frosting to occur on the glass. This can be removed as it forms by wiping with a moist cloth. We have preserved cut surface appearances in this way for as long as two months. The exposed portion of the specimen becomes markedly dried, and the tissue is not satisfactory for the preparation of microscopic sections after long preservation. Hollow organs such as the heart or the lung and various other hollow viscera cannot be preserved by this method.

For the preservation of whole organs or organs containing cavities the same temperature may be used and material preserved if the specimen is immersed in an antifreeze solution of sufficient concentration to prevent freezing of the liquid. We have used a solution of glycerin and water and have found it satisfactory. The solution is kept in the refrigerator and the specimens placed in it. With

From the Department of Pathology, Syracuse University College of Medicine.

large specimens, such as a whole liver, we have found that the central portion freezes solid while the outer portion remains fairly pliable. This may be due to partial penetration of the antifreeze solution. Small specimens remain completely pliable.

For demonstration purposes the specimens may be removed from the fluid, quickly washed to remove excess antifreeze solution and examined. They can remain out of the solution for variable lengths of time, depending on the temperature used. If hemolysis has not occurred, the specimens may be again placed in the solution and used later. We have used such material after more than three months of refrigeration, and the specimens show almost perfect-appearing surfaces. Blocks for microscopic sections from these immersed specimens may be placed directly in a 10 per cent dilution of solution of formaldehyde U. S. P. for fixation. Such sections in our experience still show satisfactory cell detail after three months' refrigeration.

Critical Reviews

DOES CHRONIC IRRITATION CAUSE PRIMARY CARCINOMA OF THE HUMAN LUNG?

MADGE THURLOW MACKLIN, M.D.
AND
CHARLES C. MACKLIN, M.D.
LONDON, ONTARIO, CANADA

Primary cancer of the lung, in common with other forms of malignant growth, is almost universally believed to originate through some form of chronic irritation, which usually sets up a chronic inflammatory process in the bronchial mucosa. The literature contains many examples of this view. Thus Davidson [1] stated: "Numerous theories have been advanced, mostly relating to the factor of irritation in some form or another of the respiratory tract, to account for this phenomenon." Simons,[2] who listed fourteen causes, found chronic irritation of the bronchial mucosa a factor common to all of them and concluded that this common factor is the cause of pulmonary cancer. Fried [3] said: ". . . it is beyond doubt that irritation is as necessary a predisposing factor in the causation of cancer of the lungs as it is in cancer of other organs." In the minds of the commentators it seems to make but little difference what the irritant is, so long as it is an irritant, and there are various types, such as mechanical, chemical, thermal, bacterial, electrical and radioactive. Often more than one type is at work on a given region, as when an area of the tongue's surface is persistently scratched by a sharp stump of a tooth (mechanical) with erosion of the epithelium and exposure of the unprotected tissues to irritants of bacterial and chemical types. The sine qua non seems to be that the irritant shall act constantly or at short intervals over a *long period*. There is no agreement as to the length of time that is required in any given case to produce a cancer; it is conceded that this factor is a variable one, and operates in association with other variables, such as age, anatomic site and predisposition. The irritant, too, must

From the University of Western Ontario Medical School.

1. Davidson, M.: Cancer of the Lung and Other Intrathoracic Tumors, Bristol, England, John Wright & Sons., Ltd., 1930.

2. Simons, E. J.: Primary Carcinoma of the Lung, Chicago, The Year Book Publishers, Inc., 1937.

3. Fried, B. M.: Primary Carcinoma of the Lung, Baltimore, Williams & Wilkins Company, 1932.

act sufficiently strongly to produce an effect in the tissue but not so strongly as to cause total necrosis of the tissue.

It seems certain also that in most if not all cases inflammation supervenes in the region undergoing exposure to the irritant. The inflammation is a reaction on the part of the tissues to the injury occasioned by the irritant. It may, theoretically at least, be regarded as protective in nature, though the means sometimes defeats the end, as in silicosis. Since chronic inflammation is a well nigh inevitable and universal concomitant of chronic irritation, the one term has often been used as synonymous with the other, and thus it is that chronic inflammation is regarded as the etiologic agent in primary cancer of the lung, without any distinction being made between the fundamental irritating agent and the tissue responses following its chronic action. So widespread is this usage with regard to chronic inflammation, indeed, that we find it convenient, in the following pages, to discuss the alleged causes of primary cancer of the lung under headings of the pathologic conditions, generally inflammatory in nature, rather than of the specific irritants. Commentators have found it difficult, too, to distinguish between chronic inflammation per se and the chronic reparative process, and it would seem that the two phenomena are so involved, one with the other, as to be practically inseparable. Thus, if we were accurate, we should ascribe the growth of the cancer to the hyperactive repair process, with its urge to increased cell multiplication, reserving to chronic inflammation or to some other factor the carcinogenic influence which first sets a cell or cells on the cancerous path.

It is our purpose in this review to bring forward data indicating *that the thesis that primary carcinoma of the lung is due to chronic inflammatory changes in the lung is unproved.* We do not feel justified as yet in denying outright that chronic inflammation causes pulmonary cancer, but we affirm that on the whole the evidence now available does not warrant such an assumption. Even the carefully selected, so-called positive evidence that is commonly regarded as supporting the thesis is by no means unequivocal.

That chronic inflammation should have been implicated as the cause of cancer is not surprising, for the two are often present in the same region. We would point out, however, that because primary cancer of the lung is not infrequently found associated with chronic inflammatory changes is no reason for assuming that it necessarily is due to these changes. Indeed this association no more justifies the conclusion that the cancer is so caused than does the circumstance of a man being found near the scene of a crime establish beyond doubt that it was he who committed the crime. His presence at the scene may have been merely fortuitous. It seems quite possible that chronic inflammation often stands in relation to primary cancer of the lung as does the innocent man at the

site of the crime. Indeed, in many cases the inflammatory changes are neither the cause nor a chance associate of the cancer but are its result.

Moreover, even if at some future time adequate proof should be forthcoming to show that in some cases chronic inflammation or irritation is the extrinsic cause of pulmonary cancer, such proof can be used only in mass data, not in individual cases. There are persons who present no evidence whatever of chronic inflammatory disease of the lungs who nevertheless show development of cancer of the lung, and, on the other hand, there are persons who, despite having suffered from inflammatory diseases of the lungs for years, never have cancer of the lung. It is impossible to say of any one case that had the chronic inflammatory change not existed cancer of the lung would not have developed.

ENVIRONMENT AND HEREDITY IN THE ETIOLOGY OF PRIMARY CANCER OF THE LUNG

As with all other cancers, the problem of the origin of primary cancer of the lung naturally divides itself into two aspects concerned with (1) supposed productive factors acting from outside the body and (2) constitutional factors inherent in the body and varying with the patient and particularly with the "strain" or line of descent. These agencies are characterized as environmental and hereditary, respectively. The environmental factors may be subdivided into two categories: (1) those that would be encountered in the course of ordinary living, which are the various chronic irritants commonly used to explain human cancer, and (2) those which have been discovered by inducing cancers in animals through the experimental use of specific chemical agents, called carcinogens. As man is not used in the experimental production of cancer, all malignant growths arising in him must come under the heading of (1) tumors induced by the chronic irritations of ordinary life or (2) tumors arising on account of constitution. These may be considered as primary poles, or extremes, useful in our mental processes in the study of the causes of cancer. Actually, the constitutional factor enters into every production of tumor, and the only point which needs to be determined with reference to it is the extent to which it is participating in any given case. So, if the environmental factor is admitted, all tumors are of dual origin.

We shall now review briefly the sources of so-called chronic irritation to determine what proof has been brought forward to sustain the thesis that chronic irritation causes pulmonary cancer in man. A large number of citations from the literature might be made in support of the idea that chronic irritation, especially in the form of chronic inflammatory change, is responsible for the genesis of primary cancer of the lung.

These would be, in practically all cases, merely opinions without any valid basis of proof. They might be encountered with an equally large number of statements questioning the role of chronic irritation in the causation of primary cancer of the lung. These, again, would be opinions, for the most part, with no proof to sustain them. They would show two things, however: first, that the theory of chronic irritation has not been accepted without question and second, that the burden of proof should rest on those who assert that primary cancer of the lung in man is caused by chronic irritation or inflammation.

ALLEGED ENVIRONMENTAL CAUSES OF PRIMARY CANCER OF THE HUMAN LUNG

Simons [2] listed a number of supposed general causes of primary pulmonary carcinoma in man, and in them he finds a common factor, namely, chronic irritation. These alleged causes should be divided into two sections: (1) those capable of being produced by pulmonary cancer (these are often found associated with such cancer because they are sometimes the results of the presence of the cancer either alone or in combination with other influences); (2) those which are not capable of being produced by cancer of the lung but which might be found associated with it in the same patient in a varying percentage of cases. With regard to group 1, it might well be that in some instances these conditions have been present before the cancer ever started, although they are not necessarily the cause of the cancer, while in others these conditions have not been present until after the cancer started and are a direct result of the growth of the tumor. We shall now discuss the conditions which belong to group 1. They are chronic bronchitis, pulmonary abscess, asthma, bronchiectasis, pleurisy, emphysema and conditions simulating pneumonia and influenza. The conditions listed in group 2 by Simons [2] are trauma, tuberculosis, pneumonoconiosis, chalicosis, syphilis, roentgen irradiations, inhalation of dust (especially from tarred roads), smoke (especially if bearing chemical irritants, as in certain of the industries), motor exhaust fumes and war gas, various industrial and occupational hazards involving the breathing of hot air (especially if bearing chemical fumes), tobacco smoke or the air of certain mines, such as those of Schneeberg, Bohemia, which contain not only irritant dusts but radioactive substances as well. It will be found that as industry expands and new chemicals are encountered, this second list of conditions which are supposed either rightly or wrongly to cause cancer of the lung will increase.

Let us review first the course of development of a cancer of the lung, so that we may see why the conditions in group 1 are so often found associated with this malady.

COURSE OF DEVELOPMENT OF TYPICAL CANCER OF THE LUNG

Although no one has followed the course of a cancer of the lung from its onset to its full expression, the following outline is regarded as probably accurate: The epithelium in some local region of the bronchus undergoes a change which makes it "malignant" to the rest of the body. At this point we are not discussing what the nature of the change is, or whether this change is occasioned by forces inherent in the cells or is brought about by extrinsic influences. The cells whose ability to differentiate into normal cells and to organize into normal tissue has been wholly or partly lost still retain their ability to grow and do grow. The growth at first is probably slow. This is the beginning of the tumor. In time the tumor causes a narrowing of the bronchus, so that an obstruction to airflow ensues, together with a handicap to the normal bronchial eliminative mechanism. This impairment of function is followed by degenerative changes in the part of the lung served by the involved bronchus. The irritation produces a cough, often nonproductive at first, of chronic character, which simulates that of chronic bronchitis. Inflammatory changes in the mucous membrane follow the damming back of the excretions normally wafted away by the cilia or removed by the peristaltoid action of the bronchus or by coughing. The cough grows worse, and blood-streaked sputum is produced. When the obstruction to the bronchus is complete, either through blocking of the lumen by the growth within or by pressure on the bronchus by the encircling growth without, there is atelectasis in the lung beyond the point of obstruction. This is followed by bronchiectasis of the bronchi in the collapse area, which dilate to make up for lost volume of lung. Another effect of the diminution in volume of lung is compensatory emphysema in the alveoli of the surrounding lung tissue. These become more dilated with coughing. An asthmatic wheeze may develop in the narrowed bronchus before it is completely obstructed.

If the center of the tumor becomes infected and undergoes necrosis, the patient may show an abscess of the lung with drainage of the pus through the bronchus. If the tumor grows extensively toward the periphery of the lung before it breaks down, the pleura becomes inflamed, and pleural effusion results. The pus may rupture into the pleural cavity, and the patient may be operated on for empyema without the cancer of the lung being recognized. The onset of the symptoms may be acute and simulate influenza or pneumonia; or the onset may be gradual, and tuberculosis may be diagnosed. In any of these steps an erroneous diagnosis may be made, and the patient may later, when the cancer is discovered, assume that the "chronic bronchitis," "influenza," "asthma" or "tuberculosis" which he was supposed to have was the *cause* of the cancer. The physician sometimes accepts at face value statements to

the effect that the patient had chronic bronchitis for some time before the cancer was recognized and that the one was therefore the cause of the other.

The course of bronchogenic cancer, as is probably true for all other cancers, can be divided into three periods: First, there is a "silent" period, which lasts from the moment of the initial change of normal cells into cancer cells up to the time when symptoms of such severity begin that the patient associates them with the onset of his illness. This stage is apparently the longest and is completely silent as far as the localized activities are concerned (though symptoms may be produced from a metastasis in some other region) but may give rise to vague general symptoms. The second, or symptomatic, period is characterized by the onset of symptoms, which progress up to the time of the diagnosis of cancer of the lung, or until death if the diagnosis is not made. This period may be relatively brief, only a few months, or at the most it may be a few years. The third period is the "diagnosed" stage and is usually the briefest; it is nonexistent if the diagnosis is not made. In most cases it lasts from the time of diagnosis of cancer of the lung until death, for relatively few patients have been operated on and cured, even for a brief time.

It is evident, then, that because of the initial silent period there is trouble in ascertaining whether any particular chronic inflammatory disease preceded the onset of the cancer of the lung. Thus bronchitis, coming before the "diagnosed" period, is thought to antedate the cancer, although the cancer existed in the silent period before the onset of the bronchitis. The symptomatic period, before cancer of the lung is recognized, during which the patient may have various pulmonary symptoms, helps to confuse cause and effect of cancer of the lung and to make patient and physician alike interpret the symptoms produced by the cancer as symptoms of conditions giving rise to the growth.

SPECIFIC SO-CALLED CAUSATIVE FACTORS OF CANCER OF THE LUNG: GROUP 1

Chronic Bronchitis.—Chronic bronchitis has been looked on by some writers as of importance in the etiology of cancer of the lung because (1) it produces chronic inflammation of the bronchial mucosa and (2) it is found with some frequency in the histories of patients with cancer of the lung. What evidence is there to support or to deny the assertion that chronic bronchitis plays a causative role in pulmonary cancer? One of the first arguments that will occur to any one is that bronchitis may be generalized in distribution, while cancer of the lung is localized. The inflammatory changes of bronchitis are widespread and peripheral as well as central, while cancer of the lung is localized and is usually hilar.

If chronic inflammatory changes induce the cancer, why does not the cancer originate diffusely over the lung?

Chronic bronchitis has no special predilection for the male sex, but cancer of the lung occurs about four times as often in males as in females. It would seem, if chronic bronchitis is a major cause of cancer of the lung, that men should be much greater sufferers from bronchitis than are women, yet no such preponderance has been mentioned in the texts on diseases of the lungs, and mortality statistics do not show more deaths from bronchitis in males than in females.

Although it might be assumed that the regeneration of the bronchial epithelium in chronic bronchitis would lead to cancer of the lung, we must ask if there is proof that it does so. First, is bronchitis followed more often by cancer of the lung than not? Second, how many patients with cancer of the lung give a history of chronic bronchitis that is of such long duration that it can legitimately be looked on as having antedated even the silent period of the cancer's growth? There are no adequate answers to either of these questions because we do not know how many persons in the population have chronic bronchitis and we have little idea of how long the silent period can be. Since most takers of clinical histories content themselves with stating that the patient had complained of chronic bronchitis, failing to give the time during which the bronchitis existed, we cannot place much reliance on reports in which it is merely stated that a certain percentage of patients with cancer of the lung have had bronchitis. First, we should have to exclude all those in whom bronchitis probably followed instead of antedated the silent period of the pulmonary cancer.

Since we know that cancer in general is slow growing, and that in men who have worked in the Schneeberg mines cancer of the lung may develop from ten to twenty-seven years after they have been pensioned off from work in the mines, we should probably have to allow a minimum of at least ten years before the cancer was diagnosed as the period during which it was probably present in the silent stage. Therefore, all patients with cancer of the lung in whom chronic bronchitis started within the last ten years before the diagnosis of cancer of the lung was made should be excluded as most probably being patients in whom the cough was either the result of the tumor or coexistent with it though not actually caused by it. Those who had a history of cough for more than ten years might possibly be looked on as patients in whom the cough antedated the cancer, although this would not inevitably be so. Second, we should then compare the latter group of patients with the general population in regard to the incidence of chronic bronchitis. If 10 per cent of the population past 40 have a history of chronic bronchitis, we need not be surprised if 10 per cent of patients with cancer of the lung

have a history of chronic bronchitis without there being any cause and effect relationship between the two. If 60 per cent of the general population have had chronic bronchitis, 60 per cent of patients with cancer of the lung may be expected to have had chronic bronchitis antedating the earliest moment of their cancer's growth without its being in any way responsible for the tumor. Unfortunately, we do not have reliable data on the incidence of chronic bronchitis in the middle-aged population, but Maxwell and Nicholson [4] remarked, "The commonest antecedent condition was chronic bronchitis, which occurred in the past history 21 times (100 cases), although when it is remembered that this condition is exceedingly common, the fact is deprived of any great significance." Most of the writers whose observations are cited to show that chronic bronchitis is a causative agent in pulmonary cancer have forgotten two facts: (1) that cancer of the lung causes chronic bronchitis in some of its victims and so explains a certain percentage of the cases in which the two conditions are found, and (2) that unless chronic bronchitis antedating the cancer is found more often in patients with cancer of the lung than in the general population of the same age and sex as these patients, its frequency in the patients with cancer of the lung, however great, is not significant. Moreover, chronic bronchitis, even including that *caused* by the cancer, does not occur in more than about 50 per cent of the patients if one strikes an average from the numerous series of cases reported in the literature.

Thus we can say that chronic bronchitis is by no means universally found in the histories of patients with cancer of the lung, that when it is found it is because the presence of the *tumor has caused the bronchitis* in a large number of the cases, that there is no way of showing that chronic bronchitis which might conceivably have antedated the tumor of the lung is more commonly found in patients with cancer of the lung in the general population and therefore that *the role of chronic bronchitis as a causative agent in the production of pulmonary carcinoma has not been demonstrated.*

Bronchiectasis.—Like chronic bronchitis, bronchiectasis is frequently a result of the presence of primary cancer of the lung, following on atelectasis caused by occlusion of a bronchus by the tumor. Hence this condition might be found either clinically or at autopsy in a large percentage of the cases of primary cancer of the lung, but it is caused by, and is not the cause of, the cancer in the majority of cases (Zacherl [5]). Because a benign tumor of the lung lasts over so much longer a period than does pulmonary cancer, a benign tumor can almost always grow large enough to occlude a bronchus before killing the patient. There-

4. Maxwell, J., and Nicholson, W. A.: Quart. J. Med. **24**:29, 1935.
5. Zacherl, S.: Wien. klin. Wchnschr. **44**:967, 1931.

fore, bronchiectasis is *always* an accompaniment of a *benign tumor of the lung* of any duration, according to Wessler and Robin.[6]

It may be, of course, that a patient who has had bronchiectasis due to some other cause may subsequently have cancer of the lung. In such a case the bronchiectasis preceded but did not necessarily cause the cancer. Many patients have bronchiectasis who never have cancer of the lung; indeed, Graham, Singer and Ballon [7] stated that in patients in whom the primary lesion is bronchiectasis "it is striking that there are but few examples of carcinoma which have developed in a bronchiectatic dilatation."

Until the proponents of the idea that the chronic inflammation caused by bronchiectasis induces cancer of the lung can show that *bronchiectasis which preceded the onset of the cancer* is followed by cancer in the inflammatory zone of the wall of the bronchiectatic cavity in a percentage of cases which is significantly higher than the percentage of cases of cancer of the lung in the population of the same age and sex distribution which is without bronchiectatic cavities, we may conclude that *no relation has been demonstrated between the chronic inflammatory changes occurring in bronchiectasis and pulmonary cancer.*

Asthma.—Asthma, although not primarily inflammatory in nature, may induce chronic thickening of the mucosa as well as of the muscle coats. It has been advanced by some authors as one of the causative agents in cancer of the lung. As in the case of the two conditions just discussed, asthmatic symptoms may result from the presence of a pulmonary cancer, and so a history of asthma preceding the diagnosis of the cancer cannot be accepted as evidence that asthma caused the cancer. On the other hand, true asthma of long duration may be found in persons in whom cancer of the lung later develops, and, again, this need not imply a causal relation between the two conditions unless cancer of the lung can be found in persons with chronic asthma in far greater proportion than in the general population. Again, we have no idea of the true incidence of asthma which antedates cancer of the lung in patients with this form of cancer, since publications based on the clinical histories give for the most part merely a statement that asthma occurred in a certain percentage of cases of cancer of the lung without giving any idea of the length of time during which the asthma had existed before the cancer was diagnosed.

From cases reported in the literature it seems that on the average about 7 per cent of patients with cancer of the lung had a history of asthma. Therefore, even if asthma is looked on as contributing to the

6. Wessler, H., and Robin, C. B.: Am. J. M. Sc. **183**:164, 1932.

7. Graham, E. A.; Singer, J. J., and Ballon, H. C.: Surgical Diseases of the Chest, Philadelphia, Lea & Febiger, 1935, p. 815.

predisposition to pulmonary cancer, it is by no means a universal cause of such cancer, and one has no evidence that it is a cause at all until one can prove that true asthma antedating beyond question the onset of a pulmonary tumor has occurred far more often in patients with cancer of the lung than it has in patients without cancer of the lung. Such proof at present is not forthcoming.

Emphysema.—Pulmonic alveolar emphysema of the degenerative type, with a barrel-shaped chest, overly large alveolar spaces and other signs, is not primarily an inflammatory change associated with regeneration of the bronchial epithelium and cannot reasonably be looked on as productive of cancer of the lung. Emphysema of the compensatory type may be an accompaniment of cancer of the lung in the later stages of the latter, after atelectasis has ensued, and is then a result not a cause of the cancer. The incidence of this condition in the general population is not known, nor is its incidence as a condition truly antedating cancer of the lung known; therefore one can make no reliable deductions as to the role which alveolar emphysema plays in causing cancer of the lung. Such a role has not been demonstrated.

Pulmonary Abscess.—This may be associated with cancer of the lung as a result of the breakdown of the center of the growth, so it is easily understood why such a history should be recorded frequently for patients with cancer of the lung. Funk [8] stated that a pulmonary abscess of obscure cause in a man past 30 should make one suspect that a malignant growth is present.

Pleural Effusion.—An involvement of the pleura in the form of dry pleurisy, a pleural effusion or empyema may be found in patients with cancer of the lung, and not infrequently it is the first symptom of which a patient complains. One of the methods of diagnosis of cancer of the lung is the centrifuging of fluid aspirated from a pleural effusion with a view to finding cancer cells in the sediment. Baker-Bates and Ellis [9] stated that a patient with pulmonary cancer may be treated surgically for empyema for some time without the underlying cause of the empyema being recognized.

Summary.—Summing up the evidence in this group of cases, we find the following conclusions to be justifiable: Because chronic bronchitis, bronchiectasis, asthma, emphysema, abscess of the lung and pleural effusion may be found as a result of the presence of the tumor in the bronchus, it is inevitable that a certain degree of association between these conditions and cancer of the lung will be found in any series of cases of pulmonary cancer. The crucial point to be proved to sustain the theory that these chronic inflammatory conditions actually

8. Funk, E. H.: J. A. M. A. **95**:1879, 1930.
9. Baker-Bates, E. T., and Ellis, G. R.: Lancet **1**:676, 1935.

predispose to cancer of the lung is that their presence actually antedating the probable time of onset of the tumor is found in significantly higher percentages of patients with cancer of the lung than of those without cancer of the lung when the two groups are kept comparable as to age, sex, and industrial occupation where there is the slightest suspicion that such occupation might be a cause of pulmonary cancer.

We have shown that such evidence has not been advanced for any one of the aforementioned conditions because there has been little attempt to separate the patients with cancer of the lung into two groups, those in whom the pulmonary conditions were a result of the presence of the tumor or at least were initiated after the tumor had started to grow, and those in whom the conditions might legitimately be interpreted as having antedated the cancer. There also has been little attempt on the part of those who affirm that these conditions predispose to cancer of the lung to ascertain the incidence of these conditions in the general population of the same age and sex as the group with cancer of the lung. The opinions advanced by the supporters of the idea that these chronic pulmonary conditions cause cancer of the lung have largely been based on the finding of these conditions in an appreciable number of patients with cancer of the lung, without regard to their incidence in the general population. We have shown that no single one of these conditions has been *proved* to be the cause of cancer of the lung. The addition of a number of uncertainties may make a larger number, but it does not add to the accuracy of the result. If none of these has been shown to cause cancer of the lung, the sum total of them has not been shown to cause cancer of the lung, nor has the common factor resident in them, chronic irritation, been shown to be conducive to the formation of tumors of the lung.

GROUP II

We now turn to the second group of so-called chronic irritations listed by Simons [2] which are looked on as causing cancer of the lung but which are not symptoms of the disease itself, although in some instances they may simulate some of these conditions.

Influenza.—In a search for previous infections of the lung which might explain the origin of primary pulmonary cancer and especially its apparently great increase since 1920, workers have suggested that influenza might be a major factor. Askanazy,[10] Winternitz, Wason and McNamara [11] and others found metaplasia in the bronchial epithelium following influenza which in some cases was almost indistinguishable from malignant growth. There is no proof that the epithelial metaplasia

10. Askanazy, M.: Centralbl. f. allg. Path. u. path. Anat. **30**:443, 1919.

11. Winternitz, M. C.; Wason, J. M., and McNamara, F. P.: The Pathology of Influenza, New Haven, Conn., Yale University Press, 1920.

following influenza went on to cancer formation, since carcinoma of the lung has developed in only a very small part of the population who had influenza in 1918 and recovered. In patients who had influenza and who have since died of some other disease there has been no higher incidence of cancerous change in the lungs than in patients who have died without having had influenza. Thus, if 50 per cent of the population now at the ages of 40 to 50 had influenza in the period from 1918 to 1920, one would expect to find that 50 per cent of the patients with cancer of the lung who are now between the ages of 40 and 50 would give a history of having had influenza. Before one may attribute to influenza a part in the causation of cancer of the lung it will have to be shown that influenza has occurred in a far greater number of persons with cancer of the lung than in persons not having cancer of the lung, and even then there would be no conclusive proof that a short attack of influenza, lasting but a few weeks, could produce such lasting changes as to initiate a pulmonary cancer. Even with specific carcinogenic agents, whose effect has been definitely demonstrated, a single application or repeated applications for a short time do not usually result in cancer; to be effective, they must be applied frequently and over a long period or must be introduced into the body in such a way that their effect is continuous.

Other arguments against the role of influenza in causing cancer of the lung are as follows: Influenza is generalized; cancer of the lung is localized. Influenza affects both sexes equally; cancer of the lung predominates in the male sex. The increase in cancer of the lung began to be noted in some countries before the 1918 epidemic of influenza.

Finally, it must be remembered that cancer of the lung may begin its symptomatic period as an attack simulating influenza from which the patient does not completely recover. There is fever, lassitude, cough and then a long-dragged-out partial recovery. The patient says that he had an attack of influenza, but what actually happened was that his pulmonary cancer began to show itself with respiratory symptoms that simulated an influenzal attack. Therefore, before deciding that a history of influenza in a patient with cancer of the lung is indicative that influenza is a causative agent, one must be sure, as with the other diseases discussed, that the disease was true influenza and that it antedated or probably antedated the onset of the cancer; i. e., there should have been a period of at least ten years between the influenza and the diagnosis of cancer. Even that might be too short a time, but anything under that is almost certain to be too brief an interval.

An average derived from numerous series of cases of cancer of the lung reported in the literature shows that in 12.4 per cent the patient gave a history of previous attacks of influenza, some of which may have

been results of the presence of the cancer rather than the cause. Kerley [12] found that although the influenza epidemic had been particularly severe in Iceland, there had been no great increase in the incidence of pulmonary tumor there. Boyd,[13] Hill [14] and Maxwell and Nicholson,[4] among others, have felt that influenza probably does not play any role in cancer of the lung. Until it can be shown that true influenza antedating the diagnosis of cancer of the lung by ten years or more occurs in the history of patients with pulmonary cancer more often than in the history of patients without pulmonary cancer one cannot accept influenza as having been proved to be an agent in producing primary pulmonary carcinoma.

Tuberculosis.—If chronic inflammatory changes are looked on as a possible or even a probable source of cancer of the lung, it is natural that tuberculosis should have been regarded as an etiologic agent because of its chronicity. Ewing [15] felt that tuberculosis was associated with cancer of the lung unduly frequently, but practically all other workers have felt that there was a very infrequent association between the two conditions. Unless tuberculosis and cancer of the lung are mutually exclusive, one should find about the same proportion of pulmonary cancers in persons dying of tuberculosis (provided, of course, that the two groups compared are kept constant in regard to age and sex) as one finds in a population not dying of tuberculosis. The necessity of the proviso is at once apparent, since the highest absolute number of deaths from tuberculosis is usually among the young, and among females. One finds, for example, in Canadian data for 1936 that 1 of every 8 men who died between the ages of 40 and 44 died of pulmonary tuberculosis. Let us assume that all of these had tuberculosis and that none had a cancer of the lung erroneously diagnosed. Also 1 of every 100 men who died at the same age died of recognized cancer of the lung. One would expect, without any significance being attached to the finding, that 1 of every 8 men dying of pulmonary cancer between the ages of 40 and 44 would also have active tuberculosis, and that 1 of every 100 men dying between the ages of 40 and 44 with tuberculosis would also have cancer of the lung.

Chiari [16] stated that from 3 to 12 per cent of patients with cancer of the lung have active tuberculosis. These figures deal with men of all ages dying of tumor of the lung. Again illustrating from Canadian data and taking only men dying after the age of 40, since pulmonary tumor

12. Kerley, P. J.: Brit. J. Radiol. **30**:333, 1925.
13. Boyd, W.: Canad. M. A. J. **23**:210, 1930.
14. Hill, R. M.: Edinburgh M. J. **41**:320, 1934.
15. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1928.
16. Chiari, H.: Klin. Wchnschr. **17**:183, 1938.

is relatively uncommon before that age, we find that approximately 3.3 per cent of men dying in 1936 in Canada died of what was diagnosed as pulmonary tuberculosis. This probably represents the minimal estimate of men dying with active tuberculosis in Canada in that year. On that basis we should expect approximately 3.3 per cent of males dying of cancer of the lung after 40 to show, in addition to the cancer, active tuberculosis, a figure agreeing with Chiari's [16] data. In countries in which there is far more tuberculosis than in Canada the proportion of patients dying of cancer of the lung who have active tuberculosis, in addition, will be far higher than 3.3 per cent; in countries in which the tuberculosis rate is lower, the proportion will be under 3.3 per cent.

It is found on examining the records of those persons with pulmonary cancer who showed active tuberculosis of the lungs at autopsy that the cancer did not grow in the wall of the tuberculous cavity, where the inflammatory changes were taking place, in the majority of instances. The tuberculosis was more often found in the lung opposite to the one in which the cancer developed than in the same lung, if one can judge from the reports in which the sites of the two diseases were given in the autopsy protocol. Or if the disease was present in the same lung, it was not in the same lobe. Even if the two processes are found in the same part of the lung, which is apparently rarely the case, this still might be due to chance association. Most authors, including Boyd,[13] Maxwell and Nicholson,[4] Chiari,[16] Funk,[8] Hill,[14] Husted and Bellmann [17] and others, have expressed the opinion that there cannot be demonstrated any causative influence on the part of tuberculosis in producing cancer of the lung. Fried [18] said: "It is of interest that the occurrence of tuberculosis and of cancer in the same person or in the same organ is rarely seen at necropsy." This observation taken together with the frequency of tuberculosis in the general population leads one to conclude that tuberculosis does not predispose to cancer of the lung.

Pneumonia.—The onset of cancer of the lung may simulate pneumonia, so that the patient may give a history of pneumonia, which he thinks was the cause of the pulmonary tumor. On the other hand, the patient may have had true pneumonia, either antedating the silent period or antedating the symptomatic period of the cancer, without there being any association between the two. Since pneumonia is by no means an uncommon disease, one would expect to find a great many in the population who have had pneumonia and recovered; therefore one would expect to find an equal percentage of patients with cancer of the lung who have had pneumonia without there being any significance in the

17. Husted, E., and Bellmann, G.: Acta path. et microbiol. Scandinav. **14**:141, 1937.

18. Fried,[3] p. 17.

fact. Thus in Canada, 5 per cent of the men dying after the age of 40 in 1936 died of pneumonia. For every man that died there were probably 3 men who recovered, since the mortality rate in pneumonia is or was at that time about 25 per cent. Therefore we can assume that another 15 per cent of men dying at that age had had the disease at some earlier date but had recovered and died of something else. Therefore we should not be surprised to find that about 15 per cent of patients with pulmonary cancer give a history of having had true pneumonia. The pneumonia may have occurred in the same lobe in which the cancer was to develop or had already developed, or it may have occurred in a different lobe or on the opposite side, as was the case with the patient reported by Graham and Singer.[19]

As to the incidence of the history of pneumonia in cases of cancer of the lung, we find the following data: Parish [20] found this history in 1 of 32 cases; Matz,[21] in 15.2 per cent of 138 cases; Hill,[14] in 10 of 107 cases; Maxwell and Nicholson,[4] in 7 of 100 cases. *These are the figures we should expect if pneumonia has no relation whatever to the production of cancer of the lung.*

War Gassing.—It is not to be wondered at that war gassing has been suggested as a possible cause of pulmonary cancer in some cases. There would seem to be only two possible ways in which war gas might cause cancer of the lung: (1) by a direct carcinogenic effect, in which case it would probably have to be applied repeatedly in small doses over a long period, since a few exposures would probably not induce cancer; (2) by the production of a chronic inflammatory condition in the lung that would be carcinogenic. The first assumption carries little weight, because it has not been shown that war gases have a specific carcinogenic effect and because there is no history of numerous repeated applications of them; the second reason is also without much value, since we have found that none of the chronic inflammatory or degenerative conditions which war gases caused, such as chronic bronchitis, emphysema, asthma, activated pulmonary tuberculosis and pulmonary fibrosis, can reasonably be linked with the development of cancer of the lung.

From data supplied by Matz[21] and by Stein and Joslin [22] on United States Veterans of the World War we see that, of 164 men with cancer of the lung, 8, or 4.8 per cent, gave a history of having been gassed in the war. Maxwell and Nicholson [4] found 4 men from among about 75 to 80 in the age range of World War veterans who had histories of having been gassed in the war and who also had pulmonary cancer.

19. Graham, E. A., and Singer, J. J.: J. A. M. A. **101**:1371, 1933.
20. Parish, T. N.: Practitioner **125**:324, 1930.
21. Matz, P. B.: J. A. M. A. **111**:2086, 1938.
22. Stein, J. J., and Joslin, H. L.: Surg., Gynec. & Obst. **66**:902, 1938.

They held that war gassing has no role in causing cancer of the lung. In order to evaluate adequately the possible role of war gassing in the production of cancer of the lung we need to know the following facts: What is the number of World War veterans living at the present time? What percentage of these were gassed in the war? In what number of World War veterans has cancer of the lung developed up to the present? What percentage of these were gassed in the war? Is the percentage of gassed men significantly higher in the group with cancer of the lung than in the group without cancer of the lung? To these questions we have no exact answers, but we have the following data: Approximately 4,750,000 men were accepted by local draft boards. Of these, 740,000 were rejected at camps because of physical and mental defects. About 87,000 died of war injuries and disease, leaving approximately 3,923,000 men who survived the war. Of these, about 75,000 had been gassed and had survived. These figures are taken from the *Military Surgeon*, of October 1936,[23] and from Matz's [21] article on war gases. Thus about 1.9 per cent of the enlisted men were gassed and survived. If the death rate in the ensuing years has been about equal in the gassed and nongassed groups, we should expect that about 1.9 per cent of the surviving veterans have been gassed. If the death rate has been differential in the two groups, that figure will be higher if the gassed veterans have not died in such great numbers, and lower if they have died in larger numbers than the rest of the veterans.

Stein and Joslin [22] reported that of 164 veterans with cancer of the lung, 4.8 per cent gave a history of having been gassed. This would appear to be two and a half times the proportion of gassed men in the veterans' group in general. But if we calculate what departure from the average percentage of gassed men one might expect in so small a sample of veterans as a group of 164, we find that, on the one hand, no gassed men might be found, and, on the other, as many as 4.1 per cent might have been gassed without there being any causal relation between the gassing and the cancer. Therefore the 4.8 per cent found is not far removed from the 4.1 per cent expected, owing to variations in samples of veterans, and we can conclude that the difference is not enough to suggest that the gassing played any part in the production of cancer. Moreover, even if gassing were conceded to have induced cancer of the lung in some veterans, it would be obvious that most such cancers developed in veterans without previous gassing (95.2 per cent) and also that in most of the men who were gassed cancer of the lung has not developed—i. e., this lesion has appeared in only several hundred among a possible seventy-five thousand.

23. Griffith, C. M.: Mil. Surgeon **79**:251, 1936.

Occupations in Relation to Primary Cancer of the Lung.—Although many attempts have been made to link the occupation of the patient with his pulmonary cancer, these attempts have been unsuccessful with the sole exception of the pulmonary cancer which occurs in the miners of Schneeberg and Joachimsthal, in Bohemia, the incidence of which is so high in comparison with that of cancer of the lung in other places and so high in comparison with the incidence among the neighbors who are not working in the mines that there seems to be little doubt that this occupation is strongly productive of cancer of the lung. It is still a disputed point as to what the carcinogenic agent is in these mines. Arsenic, radioactive emanations and air-borne molds present in the mines have been implicated.

Outdoor occupations in which dust is inhaled have been thought to be represented unduly among the patients with pulmonary cancer (Duguid [24]); indoor occupations have been found to be represented in undue numbers (Bonser [25]). Industrial hazards (Schachter [26]), laboring jobs (Brockbank [27]), chromium dust (Alwens, Bauke and Jonas [28]), iron oxide in silica dust rather than the silica itself (Campbell [29]), silica dust (Anderson and Dible [30]; Dible [31]; Klotz [32]), metal grinding, exposure to coal gas in the air or to coal tar on the skin, and the preparation and sale of tobacco (the Kennaways [33]) have all been brought forward as possible causes of cancer of the lung. In all such instances the occupation given at the time the diagnosis of cancer is made may not have been the occupation of the patient at the time his cancer developed, perhaps twenty or twenty-five years before. Moreover, one must always take into consideration the district from which the data were drawn. Thus, in an agricultural district practically all the pulmonary cancers will be found in farmers, just as will most of the gastric cancers and most of the chronic nephritis. In a district where mining is the sole occupation most of the pulmonary cancers will be in miners. In districts where every one is working in tobacco, all the pulmonary cancers will be in tobacco workers, and so on. For any one district, the effect of occupation must be determined by finding the percentage of males of certain ages working in that occupation who

24. Duguid, J. B.: Lancet **2**:111, 1927.
25. Bonser, G. M.: J. Hyg. **28**:340, 1929; **34**:218, 1934.
26. Schachter, M.: J. de méd. de Paris **52**:700, 1932; abstracted, Am. J. Cancer **19**:165, 1933.
27. Brockbank, W.: Quart. J. Med. **1**:32, 1932.
28. Alwens, W.; Bauke, E. E., and Jonas, W.: Klin. Wchnschr. **15**:1854, 1936.
29. Campbell, J. A.: Brit. M. J. **1**:755, 1938.
30. Anderson, C. S., and Dible, J. H.: J. Hyg. **38**:185, 1938.
31. Dible, J. H.: Lancet **2**:982, 1934.
32. Klotz, M.: Am. J. Cancer **35**:38, 1939.
33. Kennaway, M. N., and Kennaway, E. L.: J. Hyg. **36**:236, 1936.

have pulmonary cancers, as compared with the percentage of males of the same ages not in that industry who have such cancers. One must not compare the incidence of cancer of the lung in middle-aged males working in mines with the incidence of cancer of the lung in all the rest of the villagers, males and females, of all ages. One must try to keep the two populations compared as constant as possible with respect to all factors with one exception, namely, the factor being investigated. Thus if one is attempting to ascertain the possible effect of dusty occupations on the incidence of pulmonary cancer, one must be sure that one deals with all those exposed to the same type of dust, on the one hand, and with those who do not meet that dust, on the other. Also, the distributions of the groups compared must be the same as to age and sex. Third, those exposed to the dust must not be meeting some other factor which might also theoretically be productive of pulmonary cancer, for example tobacco smoke. Even these simple rules are seldom followed in ascertaining the role of any occupation in determining the onset of cancer of the lung.

(*a*) Silicosis: Because of the fibrosis which occurs in the lungs of persons affected with silicosis, it has been suggested that this may have a role to play in producing cancer of the lung. The changes induced by silicosis are in the connective tissue rather than in the epithelium of the bronchus, and so it is not probable that silica will prove to be carcinogenic.

Silicosis was carefully looked for by Berblinger [34] in 50 cases of pulmonary cancer, and no evidence of it was found, although silicosis is very common in his district (Jena, Germany). That last clause changes the whole aspect of the finding. Had silicosis been rare in his district, his failure to find it would have given no indication of any possible lack of relation between the two. Hill [14] found no relation between silicosis and cancer of the lung in his cases or in reviewing the literature. If the cases of pulmonary cancer are in a district where there is a high incidence of silicosis, we should find the two occurring together with some frequency; if the cases are in a district where there is little silicosis, we should expect the two conditions to be associated seldom if they are not causally related. That the conclusions drawn from the incidence of these two diseases must be carefully controlled statistically is pointed out in an editorial in the *British Medical Journal*,[35] in a discussion of the cases of Anderson and Dible.[30] These authors found a high silica content in 12 of their 70 cases of pulmonary cancer, as compared with 48 cases used as controls. The editorial points out

34. Berblinger, W.: Med. Klin. **27**:1337, 1931; abstracted, Am. J. Cancer **16**: 839, 1932.

35. Silicosis and Pulmonary Carcinoma, editorial, Brit. M. J. **2**:411, 1938.

that even if their conclusion is correct, that silica plays a role in causing pulmonary cancer, the conclusion is not justified by the material they had. It goes on to say that such a conclusion would be justified only if there were a series of almost 3,000 controls, this figure being based on the incidence of primary cancer of the lung in the general population.

Klotz [32] found pulmonary cancer in 8 per cent of his series of 50 cases of silicosis, as contrasted with 1.17 per cent of 4,500 "unselected" autopsies which he used as controls. The latter group of autopsies were apparently on patients of 15 years and upward, as it did not include the autopsies of the pediatric service. The objection to conclusions based on such material is this: In using unselected autopsies, perhaps to obtain a so-called random sample, the control material was most dissimilar from the cases of silicosis under review. Among the subjects of unselected autopsies would be a large number of females, while the patients with silicosis would be entirely or almost entirely males. Cancer of the lung is most frequent in males; therefore one would expect to find more cases of cancer of the lung among patients with silicosis than among the subjects of autopsies in general merely on the basis of unequal sex distribution. Again, the patients with silicosis are for the most part in the middle age group or old age group, for it takes years for silicosis to develop. Cancer of the lung is most frequent after 40. The subjects of unselected autopsies will include many patients in age groups well below 40, an age at which cancer of the lung is infrequent. On the basis of age distribution we should expect to find more cases of cancer of the lung among patients with silicosis than among unselected subjects of autopsies. Finally, there will be variations in the percentage of cases of pulmonary cancer from group to group of autopsy cases, even when these groups are standardized for age and sex; these variations are attributable merely to the difference in samples of the population in each case.

It may be that Klotz's [32] conclusions are correct and that pulmonary cancer is more frequent among patients with silicosis than among a group similar in age and sex but without silicosis. But we must reject his conclusions as unsound until he has compared his silicotic patients with a group of men of the same age distribution as the patients with silicosis and made due allowance for possible variations between different samples of the population.

(*b*) Asbestosis: Few cases of asbestosis associated with primary cancer of the lung have been reported (Egbert and Geiger; [36] Hornig [37]). Here again we should expect to find the two conditions associated in the same person in some cases, on chance distribution alone. Since

36. Egbert, D. S., and Geiger, A. J.: Am. Rev. Tuberc. **34**:143, 1936.
37. Hornig, F.: Ztschr. f. Krebsforsch. **47**:281, 1938.

asbestosis occurs in men of middle age, cancer of the lung may be found in these patients in a higher percentage than in the general population, but we have no proof that it is found in greater percentage when a group of men of similar age from the general population is used as the standard. The criticism that was made with respect to Klotz's data on silicosis may be made also of the data of Lynch and Smith.[38] Lynch and Smith have reported that in 2,343 consecutive necropsies over a period of twelve years cancer of the lung was found seven times, an incidence of 0.3 per cent. In the 35 cases of asbestosis, in some of which the lesions were very mild, cancer of the lung was found twice, an incidence of 5.7 per cent. Are these values significant, they asked, or is the sample of cases too small? Before such figures can be of any value, they must select from their 2,343 consecutive necropsy records only those on males, since their 35 cases of abestosis doubtless all relate to males. They must then select from these records on males only those on males in the age range of the males with asbestosis. When this is done, it will be easier to decide whether the incidence of pulmonary cancer is higher among patients with asbestosis than it is among those without asbestosis.

(*c*) Pneumonoconiosis: Other dusts besides silica are said to cause cancer of the lung. There may be much or little dust, depending on the occupations of the patients with cancer of the lung and on the district in which they live; rural patients will have little coal pigment, while residents of industrial cities like Pittsburgh will all have a great deal. South African miners have a high incidence of pneumonoconiosis but a low incidence of cancer of the lung.[2] Vorwald and Karr [39] examined the records of 15,587 persons who had long been exposed to dusty atmospheres and found that only 3 of them had cancer of the lung. He stated that the dust must be carcinogenic before its presence will induce cancer of the lung. Arkin and Wagner [40] and Berblinger [34] both found pneumonoconiosis rare in their series of cases of pulmonary carcinoma.

(*d*) Tarred Roads: Dusts and emanations from tarred roads have been said to be responsible for the increase of pulmonary cancer noted in the last two decades (Campbell [41]), but workers in Russia, where tarred roads are few, have noted the same increase in cancer of the lung as has occurred in other countries. Husted,[17] in Denmark, found that patients with primary cancer of the lung came in about the same proportion from areas where there were no tarred roads as from areas

38. Lynch, K. M., and Smith, W. A.: Am. J. Cancer **36**:567, 1939.

39. Vorwald, A. J., and Karr, J. W.: Am. J. Path. **14**:49, 1938.

40. Arkin, A., and Wagner, D. H.: J. A. M. A. **106**:587, 1936.

41. Campbell, J. A.: Lancet **1**:233, 1934; J. Indust. Hyg. & Toxicol. **19**:449, 1937.

where tarring had been carried out. Boyd [18] found no evidence that tarring had played any role in his 14 cases. Valade [42] pointed out that the conclusions which Campbell [41] reached as to the efficacy of this type of dust in causing cancer of the lung were based on the reactions of mice, which produce a high number of spontaneous pulmonary cancers, and that what might be carcinogenic for mice need not be so for men. We may conclude that there is no demonstrable proof at present that any form of dust has any role in producing cancer of the lung, for it has not been demonstrated that pneumonoconiosis is present in the lungs of patients with pulmonary cancer in any higher proportion than in a corresponding group of the general population.

Other agents, such as roentgen rays, tobacco smoke and motor exhaust fumes, have been suggested as possible causes. The suggestion that roentgen rays are a cause is almost absurd, for the probabilities are that only a small percentage of those in whom cancer of the lungs has developed ever had their chest under roentgen rays before they came for diagnosis of their cancer. Motor mechanics working in garages where there is an abundance of exhaust fumes are said to show no higher incidence of pulmonary cancer than the general population, and Campbell [43] stated that exhaust gases from internal combustion engines do not increase pulmonary cancer in mice. Tobacco smoke might be one factor in the increase of pulmonary cancer. The habit is one which permits long-continued application of the smoke to the bronchial mucosa; it is, or at least was, a habit more prevalent among men; there are two possible agents which might prove to be cancer producing, namely, heat and the derivatives of tobacco. There are those who point out that the increase in the consumption of tobacco parallels the increase in the incidence of pulmonary cancer. This may or may not have significance. If tobacco is a factor, it must be proved that derivatives of tobacco are carcinogenic or that the repeated application of heated smoke is conducive to cancer. McNally [44] stated that the tar in cigaret smoke contains enough chemical irritants to account for the increase in cancer of the lung. Roffo [45] found among a group of 5,000 cancerous women 42 who exhibited cancer in what he called the "smoke stream," namely, the lips, tongue, jaws, larynx and pharynx. All of these women were heavy smokers. He found that the products of burnt tobacco produced carcinoma when painted on rabbit ears, and he feels that there are probably many aromatic substances in tobacco that are carcinogenic. His findings are suggestive. But the increase

42. Valade, M. P., cited in Carcinogenesis in the Lung, Annotations, Lancet **1**:521, 1939.

43. Campbell, J. A.: Brit. J. Exper. Path. **17**:146, 1936.

44. McNally, W. D.: Am. J. Cancer **16**:1502, 1932.

45. Roffo, V. A. H.: Deutsche med. Wchnschr. **63**:1267, 1937.

in cancer of the lung should be much greater among females than among males, since smoking has increased among women far more rapidly in the last two decades than it has among men. Data published by the Metropolitan Life Insurance Company [46] show that the incidence is not greater among females. The rates among the industrial policyholders of 45 to 74 years of age show that in 1917 the rate for pulmonary cancer among females was 2.5 per hundred thousand. This rose to 8 per hundred thousand in 1938, an increase of 220 per cent. The rate for cancer of the lung in males rose in the same period from 3.2 to almost 23 per hundred thousand, an increase of more than 600 per cent. Their conclusion is that it appears doubtful that smoking is a factor in causing cancer of the lung.

Controlled observations over a longer period are necessary to settle this point. Investigators must not rest content with finding that a high percentage of patients with pulmonary cancer are tobacco smokers; they must find that the percentage of smokers in this group is significantly higher than that in the general population of a similar age and sex distribution.

When we review all the theories on the cause of pulmonary cancer, we find that not one supposed cause has been *proved* to be a real cause and that none of the alleged causes has been investigated with sufficient statistical accuracy to enable the observer to pass an opinion carrying any weight. No one cause, no sum of causes, no common factor in them, namely, chronic irritation through inflammation of the bronchial mucosa, has been demonstrated to occur with any greater frequency in patients with cancer of the lung than in a group from the general population of comparable age and sex distribution at a time sufficiently long before the onset of the cancer that it may be interpreted as antedating this rather than following it. Naturally, any condition which is a sequela of cancer of the lung may be found more frequently in patients with pulmonary cancer than in persons not affected with this type of cancer. Such a condition when obviously the result of cancer of the lung must be excluded from a discussion of the latter's causes.

(*e*) Schneeberg Miners: The high frequency of cancer of the lung among the miners of the Schneeberg and Joachimsthal valleys has been recognized for many years. It seems that this occupation is one which does enhance the incidence of pulmonary cancer beyond that known elsewhere and apparently beyond that of the neighbors of the valleys who do not work in the mines. Thus Pirchan and Sikl [47] stated that 12 of 19 Schneeberg miners dying in 1929-1930 had cancer of the lung at autopsy. Of 12 miners dying in 1932, 9 had a *clinical* diagnosis of

46. Cancer of the Lung a Growing Health Problem, Statist. Bull. Metrop. Life Ins. Co. **20**:7, 1939.

47. Pirchan, A., and Sikl, H.: Am. J. Cancer **16**:681, 1932.

pulmonary cancer. Sikl [48] reported similar figures for the miners of Joachimsthal. Of 15 miners dying in eighteen months, autopsies were carried out on 10; of these, 8 had carcinoma of the lung. The average age of these men was 49, and the average period of exposure in the mines was seventeen years. This incidence of cancer of the lung has not been equaled anywhere else in the world. The cobalt, the arsenic, the dust, the radioactive emanations all have been incriminated. Sikl [48] stated that a chemical analysis of the lungs showed no trace of important foreign elements. The test for radioactivity was negative, although he felt that radioactive emanations in the mines may have a part in producing pulmonary cancer. Maisin [49] quoted de Laet as saying that in Belgium pulmonary cancer has not developed in workers in radium although they wear no masks and are covered with and breathe the dust of the works all the time. However, the plant had been in operation only twelve years at the time of de Laet's report.

Of interest in this connection are Martland's [50] findings in workers with radium paint. The girls who died of osteogenic sarcoma were exhaling radioactive air, as shown by tests, yet none of them presented cancer of the lung. A whole series of questions to which we have as yet no answers, and may never have, arise. If the radioactive emanations in the air in the Schneeberg mines are responsible for cancer of the lung, why did the radium paint workers in the United States die of bone and not lung tumors, especially as it was shown that the lungs were exposed to radioactive emanations? May it be that there is an inherited factor for cancer of the lung in the Schneeberg miners which has been increased through long-continued inbreeding in these rather isolated mountain valleys with a small population and that the carcinogenic agent in the mines (whatever it may be) enhances an already present tendency toward pulmonary cancer? Is it possible that, taken in small doses, the radioactive substance induces pulmonary cancer, which may have a long latent period, whereas radium taken in presumably larger quantities, as is the case when radium paint is swallowed, may induce bone tumors, with a shorter latent period of development? Thus these victims would die of osteogenic sarcoma before they had time to develop pulmonary cancers. Certain it is that although the miners have a very high incidence of cancer of the lung they do not have a high incidence of those chronic pulmonary conditions which are supposed to predispose to cancer of the lung. Thus Pirchan and Sikl [47] said that of the 9 men with clinically diagnosed cancer of the lung, not one had a history of chronic pulmonary disease that was not directly related to the onset of the cancer.

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48. Sikl, H.: Ztschr. f. Krebsforsch. **32**:609, 1930.

49. Maisin, J.: Cancer, Bruxelles **11**:111, 1934; abstracted, Am. J. Cancer **28**: 164, 1936.

50. Martland, H. S.: Am. J. Cancer **15**:2435, 1931.

Throughout this discussion we have repeatedly emphasized that it makes no difference how great is the percentage of patients with cancer of the lung who point to any particular disease in their past history, provided that a similarly great percentage of the general population has had the same disease. Thus if the cancerous patients come from a region in which the whole population has had measles, every patient with cancer of the lung will give a history of measles. If 90 per cent of the population past 40 have had tuberculosis, 90 per cent of the patients with cancer of the lung will probably give a history of tuberculosis. If the main occupation in the district is farming, most of the patients with cancer of the lung will be farmers, but if mining is the main industry, most of the patients with cancer of the lung will be miners. This fact has been little appreciated by those who argue that because 25 or 50 or 75 per cent of patients with cancer of the lung give a history of a particular disease that disease must be carcinogenic; hence we shall try to present this matter more in detail at this point.

In the accompanying chart the first rectangle represents a theoretic population. One fourteenth of them (black bar at the top) is characterized by having a certain disease, X. The subsequent rectangles represent those portions of the population which have had some other condition. Thus in rectangle 2 we might indicate those who had had, for example, chronic bronchitis; in 3 those who had had influenza; in 4, those with arteriosclerosis, and so on. We will assume that none of these groups overlap. Now it will be seen that one fourteenth of the population in rectangle 2 also has disease X, the same proportion as was present in the general population. This is true of the groups represented in rectangles 2 to 6. The group in 7 shows a much higher concentration of X patients than do the other groups, while the group in 8 has fewer X patients than has the general population. From such a diagram one would be justified in looking on the group in 7 as probably peculiarly susceptible to disease X, while those in 8 would be considered relatively immune. We are assuming in this example that all the groups are comparable as far as sex, age and other significant factors are concerned.

Now let us arrange these same data in rectangle 9, which represents the population affected with X, which is one fourteenth of the total population. Here we see that three groups each have a fourth of the patients with disease X. These are 2, 3 and 7. Group 4 has an eighth; group 5, one sixteenth, and groups 6 and 8, each a thirty second of all X patients. If we looked at this column without viewing the data in columns 1 to 8, we should be very likely to conclude that groups 2, 3 and 7 are equally susceptible to X and that groups 6 and 8 are the most immune to this disease. The latter conclusions would, of course, be wrong, as the data in the rest of the diagram clearly show.

It is in the latter form that the data dealing with the cause of pulmonary cancer are usually presented. We are told that a certain percentage of patients with cancer of the lung give a history of chronic bronchitis or of influenza or of some other condition, and if this percentage is high, it is assumed that the disease named is an important

1. The general population is represented in rectangle 1. Those with a history of a disease which we will call X are shown by the black bar at the top. This is one fourteenth of the total area of rectangle 1. All percentages in this figure are of course theoretic and are used merely for purposes of demonstration.

2. This rectangle represents the proportion of the total population, namely, 25 per cent, which has had chronic bronchitis. Note that one fourteenth of this group also have X.

3. This rectangle represents those in the population, namely, 25 per cent, who have had influenza. One fourteenth of them have X.

4. This rectangle represents those in the population who have arteriosclerosis, namely, 12.5 per cent. One fourteenth of them have X.

5. This represents the part of the population which has asthma, namely, 6.25 per cent. One fourteenth of them have X.

6. This represents the part of the population with a history of war gassing, namely, 3.12 per cent. One fourteenth of them have X. Because X has developed in the five groups 2 to 6 in the same proportion as in the general population, we cannot assign a specific role to any of these conditions in the production of disease X.

7. This is the part of the population who are miners of radioactive ores, namely, 3.12 per cent. Here the incidence of X is eight times as great as that found in the general population or in the five preceding groups of the population. It appears that some factor associated with mining of these ores is productive of X. But because not all miners have X, and because many who are not miners do have X, other factors besides the mining of these ores enter into the cause of X.

8. This is the part of the population which has a history of tuberculosis, namely, 25 per cent. The incidence of X is much lower here than in the general population, as if some factor inhibiting the development of X was present in this group.

9. This rectangle shows the part of the population in which X develops. One fourth of these have had bronchitis, another fourth influenza, another fourth exposure to radioactive ores. One eighth have arteriosclerosis, one sixteenth have asthma, one thirty-second have had tuberculosis, and one thirty-second have been war-gassed. Data in the latter form are usually used to determine the importance of any specific disease as a cause of cancer of the lung. It is assumed that all the groups in the rectangles 1 to 9 are of the same age and sex distribution and that no group in any rectangle overlaps that in any other.

causative factor in producing carcinoma of the lung. If the percentage is low, it is assumed that the disease has no significance in the causation of cancer of the lung. The diagram in figure 1 shows that it is only when the disease in question is found in a much higher percentage of the group with pulmonary cancer than of the general population that one can consider that it has an etiologic role in producing pulmonary cancer.

But even this is not enough. It is necessary that the group in the general population which we are using as a control for the pulmonary cancer population be comparable to the latter in all respects except that the general group does not have cancer of the lung; otherwise one might find that the group with cancer of the lung had the condition in question in a higher percentage than had the general population without there being any significance in that fact. For example, some of the veterans of the World War are asking for compensation for cancer of the lung, which they claim was brought on by the chronic pulmonary diseases which they contracted through exposure encountered during military service. Now how might one go about settling the justice of such a claim? One might say, "The incidence of pulmonary cancer among war veterans is much higher than among the general population, therefore it must have been the hardships of military service which caused this increase." (We are assuming for the sake of the argument that it is more frequent among war veterans.) But the war veterans are all males, and the general population holds more females than males. Hence an adjustment for sex would have to be made so as to make both groups comparable. Next, the average age of the war veterans lies between 45 and 60, while the age of the general population will range all the way from birth to 100 years. The two groups must be made comparable as far as age is concerned. Then one might argue that although the hardships of military service did not actually cause pulmonary cancers they caused these lesions to develop much earlier, because the age at which the cancers were developing in the veterans was much younger than that of the patients with such cancers in the general population. But again the age distributions in the two groups are different, the ages of the veterans being concentrated in the range from 45 to 60, while the general population has those under 45, among whom pulmonary cancer is not frequent, and those from 60 on, among whom it is frequent. Hence only those among the veterans who were destined to get cancer of the lung at younger ages would be included in the veteran group. Those in whom it will develop after 60 are not included, since the veterans have not yet reached 60 years and over. In this way the average age at which pulmonary cancer developed in the veterans would be less than that at which it develops in the general population.

SUPPOSED ENVIRONMENTAL FACTORS: A GENERAL DISCUSSION

The introduction of a consideration of experimental work into a discussion of carcinoma of the human lung may seem irrelevant, but it is not really so. For a few decades the experimental production of carcinoma by so-called chronic irritants lent strong confirmation to the idea that chronic irritation was the sine qua non of all cancer. Thus when no evident chronic irritant was found, it was assumed that it was present anyway, as witness the citations at the beginning of the paper. The evident irritants were mechanical, thermal, chemical and bacteriologic agents. Sometimes it was stated that it was not the irritant but the chronic inflammatory changes set up by it that produced the cancer. In recent times, however, we have come to look on carcinogens as involving more *specific* agents. Certain viruses, such as that of Rous, have been shown to be the cause of certain definite animal tumors. Specific chemical bodies, such as dibenzanthracene and the estrogens, have been shown to produce carcinoma on repeated application. Much depends, apparently, on the chemical formula; comparatively minor alterations in the structure of the compound mean wide alterations in the carcinogenic power. A large literature has grown up around these carcinogens (Cook,[51] Andervont [52] and others).

It has been found experimentally that not all chronic irritants produce cancer and that some of the highly carcinogenic agents are not irritating, that what may be carcinogenic for one animal need not be for another animal or even in another strain of the same animal, and finally that what is carcinogenic for one organ in an animal need not be productive of cancer in another organ of the same animal.

Let us illustrate these points briefly. Some marked irritants, such as mustard gas, are not cancer producing although they cause intense inflammatory reactions (Visser and ten Seldam [53]). Again some highly carcinogenic chemicals are nonirritating when applied to the skin (Andervont [52]). Tar painted on the skin of rats did not produce cancer of the skin, while if painted on mice or rabbits it did cause such cancer. The same tar painted on mice differing in their genetic susceptibility produced cancer of the skin in highly susceptible mice and failed to produce cancer in highly resistant mice (Kreyberg [54]). It is of interest

51. Cook, J. W.: Proc. Roy. Soc., London, s.B **111**:485, 1932.

52. Andervont, H. B.: Effect of Bacterial Products on Growth of Malignant Tumors, in A Symposium on Cancer, Madison, Wis., University of Wisconsin Press, 1938, p. 54.

53. Visser, J., and ten Seldam, R. E. J.: Geneesk. tijdschr. v. Nederl.-Indië **77**:3092, 1937.

54. Kreyberg, L.: Genetic and Constitutional Aspects of Spontaneous and Induced Tumors, in A Symposium on Cancer, Madison, Wis., University of Wisconsin Press, 1938, p. 183.

that cotton mule spinners suffer from cutaneous cancer due to the constant spraying of the skin with liquid petrolatum, whereas they do not exhibit cancer of the lung in any increased percentage although they constantly inhale air in which the same oil is present in the form of fine spray (Simpson[55]). Chimney sweepers had a high percentage of cancer of the skin, yet the constant inhalation of an atmosphere containing the same carbon particles as caused cutaneous cancer did not lead to cancer of the lung. Injections of estrogens are productive of cancer of the breast and of the uterus in mice but not of cancer of the gastrointestinal tract or of the lung.

Therefore it can be stated that before any condition, such as chronic pulmonary disease, can be regarded as producing cancer it must be proved beyond doubt that (1) the condition is capable of producing cancer apart from any ability to cause chronic inflammatory change, (2) that it is capable of producing cancer in the animal under discussion and (3) that it is capable of producing cancer in the specific organ in question. Merely to show that a condition induces chronic inflammatory changes in an organ is no longer sufficient, for it is known that chronic inflammatory changes can exist for long periods without producing cancer and also that cancer can occur with no reasonable evidence of preceding inflammation—for instance, in retinal blastoma shortly after birth.

PROBABLE ROLE OF HEREDITY IN PULMONARY CANCER

Although specific carcinogens can produce cancerous changes, are the conditions mentioned earlier in the paper shown to be carcinogenic? It has been assumed that they may be because one has no other explanation of pulmonary cancer if one insists on the presence of extrinsic factors as the sole causative agents of all cancers. But cancer is a problem of failure of differentiation, of organization and of growth; differentiation, organization and growth are problems of the inherent makeup of the cell itself and of its ability to utilize certain chemicals in its metabolic processes. Therefore one should not overlook the fact that "idiopathic" alterations in the inherent makeup of the cell may be responsible for cancerous changes without the intervention of any carcinogenic agent. In other words, the cancer may arise through hereditary causes. It is known that under the influence of hereditary determiners descendant cells of the original zygote differentiate into cells with quite widely differing potentialities. Absence or alteration of these determiners for normal differentiation may also be hereditary. Thus the alterations necessary for changing a normal cell into a cancer cell may be under the aegis of determiners located in the germ plasm.

55. Simpson, S. L.: Quart. J. Med. **22**:413, 1929.

This alteration sui generis may come about through two different mechanisms. One may be a somatic mutation, i. e., a sudden departure from the normal line of cells from which the altered cell sprang, which departure then becomes hereditary for all cells descended from that original mutating cell. That such a mutation should occur would be wholly unexpected, since there was no factor in the original fertilized germ cell from which the individual came which played a role in determining the occurrence of the mutation. The patient with cancer of the lung in this case would not have come from a strain in which factors in cancer of the lung were inherited, nor would he be liable to have more offspring in whom cancer of the lung would develop than a person without cancer of the lung.

The second mechanism would be one in which the tendency to alter into a cancerous cell would have been inherited, being due to factors resident in the fertilized germ cell. Just as there are inherited factors which guide the destiny of the entodermal cells which form gut, then bile duct, then liver, so that this development proceeds normally in an orderly fashion at definite times in the life of the embryo, the times differing depending on whether the embryo comes from a mouse, a dog or a human ovum, and just as there are inherited factors which permit cartilaginous development at the epiphysial lines for so many years in the human humerus but for a different number of years in the femur before the cartilage cells cease proliferating and allow bone growth to cease, so there may be inherited factors which permit cells in a specific location at a specific time in the life history of the individual to alter and become cancer cells.

What proof have we that these last two mechanisms are possible and that not all cancer nor indeed all pulmonary cancer needs be interpreted as arising through the agency of extrinsic carcinogenic factors? First, with the single exception of the pulmonary cancer of the Schneeberg and Joachimsthal miners, we have no adequate proof that cancer of the lung in man is caused by extrinsic factors. This is negative evidence, but it should be of value in forcing us to hunt for other causes of human pulmonary cancer before we accept all pulmonary cancer as being due to external agents. For the idea that sporadic somatic mutations or hereditary alterations are frequently the cause of cancer of the human lung we have the following indirect additional evidence. The vast majority of such cancers in man appear to rise from one point of origin, as would be expected were a somatic mutation the cause, whereas if external agents acting on the lung were responsible we should expect that the tumors would be multiple and over both lungs. Thus tumors induced in mice by hydrocarbons are not bronchiogenic as in man, but subpleural, and may be multiple rather than

single, and may be bilateral. Second, we know that mutations do occur in the germ cells and that the cells with such mutations are then propagated as a new line of individuals. There is no reason to suppose that mutations are limited to germ cells alone and that such mutations could not occur in any somatic cell in the body.

Have we any evidence that definite hereditary factors may be present in the germ cell which cause cancer of the lung to appear at a definite time in the life cycle of the individual? As far as cancer of the lung is concerned we have no human pedigrees that would prove it to be inherited, and again the evidence must be indirect. Cancerous alterations of cells have been found to be inherited in strains of mammals in which experimentation is possible. The type of cancer produced by the strain is more or less constant. Thus there are strains with a high incidence of pulmonary tumors (Lynch [56]), others with a high incidence of mammary tumors and others with a high incidence of hepatic tumors. There are strains of mice in which several types of tumors may be capable of developing but which tend to die of the type developing first, thus obscuring the other cancerous potentialities. Since man biologically is much more closely *identified with* other mammals than he is *differentiated from* them, it would be reasonable to suppose that if cancer shows dependence on hereditary factors in mice the same will be true for man, although the actual mode of inheritance may differ widely in the two species.

In rectal, gastric, mammary and uterine cancer in man we see good evidence that related members of a family tend to resemble each other more closely in the production of these tumors at a definite time in their life than do unrelated members of a community (Macklin [57a]). This suggests that hereditary factors play a predominant role in many if not in all of these tumors. Cancer of the lung was seldom recognized before 1900. Hence, even if it were inherited and transmitted according to a simple scheme so that one would frequently encounter familial cases, we should not have many records of familial instances, owing to the fact that pulmonary cancers in the preceding generations remained undiagnosed.

Finally, we may use the evidence furnished by tumors in twins (Macklin [57b]). True we have no records of pulmonary cancers in twins, but since cancer of the lung is like all other cancers except that it occurs in the lungs instead of elsewhere, we may not be assuming too much when we use tumors in twins to show that cancer and hence pulmonary cancer may be inherited. If one examines records of can-

56. Lynch, C. J.: J. Exper. Med. **54**:747, 1931.

57. Macklin, M. T.: (*a*) Familial Incidence of Cancer, in a Symposium on Cancer, Madison, Wis., University of Wisconsin Press, 1938, p. 32; (*b*) J. Heredity **31**:277, 1940.

cers in monozygotic and dizygotic twins, one finds the following facts: Monozygotic twins, with an identical inheritance, resemble each other far more often in both having a cancer and having it in the same organ and at about the same time of life than do dizygotic twins, who may have with respect to cancer either an identical or a different heredity. This is true even when the dizygotic twins studied are all of the same sex, thus eliminating differences in environment due to occupation which are present when the twins are of the opposite sex. This would indicate that although somatic mutations may be responsible for some cancers they are probably not responsible for all, for it would be very rare for identical twins to have an identical somatic mutation in the same organ at about the same age were that mutation not under the guiding influence of heredity.

To sum up, then, it would seem that the so-called chronic irritants supposed to be responsible for cancer of the lung have not been proved to be causes of such cancer, since (1) many have cancer of the lung who are not exposed to these chronic irritants, while (2) many exposed to them never have cancer of the lung. Until such proof is forthcoming, investigators are closing their minds to other, perhaps fruitful possibilities by insisting that because specific carcinogens are responsible for cancer of the lung in some instances they must be in all. Such an attitude is no more reasonable than to state that because diabetes develops in an animal from which the pancreas has been removed, all diabetics are without a pancreas. Sporadic mutations and hereditary influences may and most probably do play a large role in causing pulmonary cancers as well as other types of malignant growth.

SUMMARY

In conclusion, then, we may say that none of the specific diseases or conditions causing chronic inflammation of the lungs can be said to have been proved to be causes of cancer of the lung. They have not been shown to be more common in patients with pulmonary cancer than in a group of the general population of similar age and sex, and until a *significant* difference can be found between their incidence in those with pulmonary cancer as compared with those without pulmonary cancer their causal relation to this type of cancer will remain purely speculative. This, of course, refers to chronic inflammatory diseases which have antedated the cancer by a sufficient number of years so that they can be excluded from being interpreted as the result, not the cause, of the cancer.

Chronic irritation, especially in the form of chronic inflammatory disease, as a cause of pulmonary cancer has been assumed; the idea has been copied from text to text, repeated from author to author, with

little critical analysis of its possible role. Somatic mutations and hereditary factors must be considered and their probable role in causing cancer of the lung recognized. True carcinogenic agents for the production of cancer of the lung will no doubt be found as modern industry expands its use of chemicals. But, as some types of cancer will develop in one seventh of men over 40 and since about one fifth of all cancers in males are in the lungs, one thirty-fifth, or about 3 per cent, of all men over 40 will die of this type of cancer. There may be an increase in the incidence of cancer of the lung in any industry beyond the maximal rate for cancer of the lung in the general population, but one can never state that any patient would not have had cancer of the lung had he not been in that occupation. Thus even with the presence of carcinogenic agents admitted, their role in causing cancer of the lung cannot be proved in any individual case, nor can they be said to be 100 per cent effective, since a certain percentage of persons exposed to the carcinogenic agents would have had pulmonary carcinoma even without this exposure. The effectiveness of any carcinogenic agent in producing carcinoma of the lung can be judged only by the *increment of increase* of carcinoma of the lung in large groups, ***not by its total incidence.*** It is therefore impossible to assign to any extrinsic factor an absolute role in carcinogenesis in any individual case.

Notes and News

University News, Promotions, Resignations and Appointments.—George W. McCoy retired from the United States Public Health Service on June 30, after forty years of active service. He served as director of the Hygienic Laboratory, now the National Institute of Health, from 1915 to 1937.

Nicholas Kopeloff has been appointed assistant professor of bacteriology in the College of Physicians and Surgeons of Columbia University.

William H. Woglom has been appointed acting executive officer of the department of cancer research at the College of Physicians and Surgeons of Columbia University.

The board of scientific directors of the Rockefeller Institute for Medical Research announces promotions and appointments effective on or after July 1. The promotions are: Associate member to member—Duncan A. MacInnes, Richard E. Shope and Wendell M. Stanley; associate to associate member—Moses Kunitz and Alfred E. Mirsky; assistant to associate—Douglas A. Macfadyen and William Trager; fellow to assistant—Armin C. Braun, Malcolm S. Ferguson, Birdsey Renshaw and William C. Spring Jr. New appointments are as follows: assistants—Gail L. Miller, Howard A. Schneider and Armine T. Wilson; fellows—James A. Baker, Claude A. Knight Jr., Kermit W. Kreitlow, Margaret R. McDonald and Walter Schlesinger.

A. R. Dochez, John E. Borne professor of medical and surgical research in the College of Physicians and Surgeons of Columbia University, has been appointed chairman of the department of bacteriology.

Edward J. Stieglitz, formerly associate professor of clinical medicine at Rush Medical College, of the University of Chicago, is working on problems of aging, at the National Institute of Health, Washington, D. C.

George Blumer, clinical professor of medicine in Yale University since 1920, has retired from active practice. Dr. Blumer was formerly professor of pathology in Albany Medical College and in Cooper Medical College, San Francisco, and was Ely professor of theory and practice of medicine at Yale from 1906 to 1920.

Recent Deaths.—Fritz Schiff, bacteriologist at the Beth Israel Hospital, New York, died on July 30, at the age of 51 years. Dr. Schiff came to New York in 1936 from Berlin. He made important contributions to bacteriology and to the knowledge of blood grouping.

John Gerald FitzGerald, professor of hygiene and preventive medicine at the University of Toronto and director of the School of Hygiene and of the Connaught Laboratories, died on June 20, at the age of 57 years.

Joseph W. Schereschewsky, medical director of the United States Public Health Service, pioneer in the analysis of cancer death rates and in the organization of cancer research and anticancer state programs, died July 11, 67 years of age.

John A. Lanford, assistant professor of pathology and bacteriology in Tulane University of Louisiana and pathologist to the Touro Infirmary in New Orleans, has died at the age of 59 years.

Hans Zinsser, professor of bacteriology and immunology at the Harvard Medical School, died on September 4 in his sixty-second year.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Chloroform Injury of the Liver Increases as Protein Stores Decrease. L. L. Miller and G. H. Whipple, Am. J. M. Sc. **199**:204, 1940.

The injury of the liver associated with chloroform anesthesia increases in extent as the protein stores of the body are depleted. In a protein-depleted dog chloroform anesthesia lasting fifteen to twenty minutes is frequently fatal, causing extensive hyaline necrosis of the liver and the typical picture of chloroform poisoning. Chloroform anesthesia lasting twelve minutes will cause moderately severe hepatic injury in a protein-depleted dog. Protein depletion is effected by giving low protein diets alone or combined with bleeding or plasmapheresis. Control experiments in dogs without protein depletion indicate that ninety minutes of chloroform anesthesia can be tolerated with little injury to the liver.

In the depleted dog xanthine given subcutaneously or aminoacetic acid given by mouth do not protect, but a single large protein feeding thirty-six hours before chloroform anesthesia may protect against hepatic injury. In contrast to the non-depleted or the anemic dog these protein-depleted dogs show no increase of urinary creatine after hepatic injury. The protein-depleted dog compared with the non-depleted dog after chloroform injury to the liver shows relatively little increase in total urinary nitrogen. There is a conspicuous increase in the excretion of uric acid in these depleted dogs after chloroform injury to the liver, perhaps due in part to impairment of liver function and disturbance of its normal capacity to convert uric acid to allantoin.

From Authors' Summary.

Arsphenamine Injury of the Liver Modified by Diet. W. J. Messinger and W. B. Hawkins, Am. J. M. Sc. **199**:216, 1940.

When dogs are given arsphenamine in doses of 0.03 Gm. per kilogram or higher, injury of the liver results to a greater or lesser degree, depending on the diet fed. Protein is found to be most effective in protecting dogs against arsphenamine injury of the liver. On a protein diet the injury to the liver is trivial and promptly repaired. A carbohydrate diet also is beneficial but is not as uniformly protective, and the injury of the liver may be somewhat greater. Fat has proved to be deleterious, as the arsphenamine-treated dogs show marked progressive jaundice and severe hepatic injury and may become intoxicated to the point of death. In certain instances dogs that had been protected by protein or carbohydrate showed a progressive increase in the icteric index when changed over to the fat diet, even though no additional arsphenamine was injected. When fat-fed dogs that showed severe intoxication due to arsphenamine injury were switched to protein or carbohydrate diets, they immediately recovered from the intoxicated state, and the icteric index decreased. Subsequent injections of arsphenamine caused no ill effects as long as the dogs remained on either the protein or the carbohydrate diet.

From Authors' Summary.

Experimental Botryomycosis. P. Kimmelstiel and C. A. Easley Jr., Am. J. Path. **16**:95, 1940.

Foreign body granulation tissue was experimentally produced by implanting fish bones in the intestinal walls of rabbits. If the implantation was accompanied

by a perpetual infection with small numbers of staphylococci, granules were formed which were identified as botryomycotic granules. Botryomycosis, therefore, is believed to be due to continuous invasion of the tissue by staphylococci. Foreign bodies, such as bones, provide a way of perpetuating the infection.

FROM AUTHORS' SUMMARY.

EXPERIMENTAL DISSEMINATED ENCEPHALOPATHY IN THE MONKEY. A. R. FERRARO and G. A. JERVIS, Arch. Neurol. & Psychiat. **43**:195, 1940.

Ferraro and Jervis inoculated monkeys intramuscularly with extracts or suspensions of human brain. The animals showed forced position of the head, ataxia, tremors, nystagmus, spastic paresis and partial ophthalmoplegia. The course was progressive. There were disseminated circumscribed perivascular cell accumulations in the central nervous system, especially in the centrum ovale, cerebellum and pons. Striking were giant cells with many nuclei and foamy cytoplasm. Foci of demyelinization (one involved almost the entire white matter of the cerebellum) were present as the result of confluence of smaller foci. In addition, there were foci in which demyelinization was only partial and in which no connection with blood vessels could be observed. The authors suggest that the changes were due to the action of the antibodies which develop from injection of heterologous brain tissue.

GEORGE B. HASSIN.

PROSTATIC SECRETION. C. HUGGINS and others, J. Exper. Med. **70**:543, 1939.

A simple isolation of the prostate made possible quantitative collection of prostatic secretion in dogs over periods of months. The secretory stimulant was pilocarpine, and two similar amounts injected six hours apart gave a smaller amount at the second testing, suggesting an effect of fatigue. The prostate was not absolutely refractory, since doubling the amount of the alkaloid injected at the second test increased the volume to equal or to exceed the preliminary secretion. The depression effect had disappeared at twenty-four hours. In normal dogs the secretory curves were essentially regular, with only occasional prolonged rises or depressions. The amount of the secretion did not bear a direct relation to the weight of the gland in the adult dog. The germinal epithelium of the testis underwent atrophy during the first few weeks of cage life while the prostatic secretion was maintained, showing that the atrophy was differential and did not involve the cells producing the androgen. The atrophy was reversible, and all dogs kept for more than four months showed restoration of the germ cells. Atrophy of the germinal cells occurred in a few dogs, with cessation of prostatic secretion, and persisted for many weeks, but with final recovery. Removal of the adrenal glands, producing adrenal insufficiency, did not produce sterility. The distribution of electrolytes in the prostatic secretion differed from that in the serum transudate system, although the concentration of osmotically active substances was the same, being made up almost entirely of sodium and chloride. The distribution was not affected by the different physiologic procedures used in this study. Protein concentrations were less than 1 per cent. The rate of prostatic atrophy following castration was determined; cessation of secretion occurred in seven to twenty-three days. The restoration of prostatic fluid in castrate dogs following daily injections of testosterone propionate followed a smooth curve to form a plateau which was interrupted occasionally by prolonged elevations with return to the established level. The prostate having been reconstructed, the dosage of androgenic material injected could be greatly reduced without causing a decrease in secretion. Ablation of the thyroid and parathyroid glands had no significant influence on prostatic secretion. Hyperthyroidism caused a secretory depression interrupted with returns to normal levels.

FROM AUTHORS' SUMMARY.

DIETARY PROTEIN AND NEPHROTOXIC NEPHRITIS. L. E. FARR and J. E. SMADEL, J. Exper. Med. **70**:615, 1939.

Acute nephritis of medium severity, affecting both glomeruli and tubules, was produced in rats by injections of an antiserum for rat kidney, given on three consecutive days. The course of the nephritis was markedly influenced by the type of diet which was fed. Rats tended to recover promptly from the induced nephritis when a low protein–high carbohydrate diet was given. On the other hand, in nephritic rats maintained on a medium protein diet the nephritis almost invariably became chronic, and half the animals died of renal insufficiency during the ten and one-half months of observation. Finally, none of the rats which received a high protein–low carbohydrate diet recovered from the acute renal injury; all acquired chronic progressive nephritis, and the majority died of renal failure after some months.

FROM AUTHORS' SUMMARY.

EXPERIMENTAL HYPERTENSION. C. GERBI, B. B. RUBENSTEIN and H. GOLDBLATT, J. Exper. Med. **71**:71, 1940.

The consumption of oxygen by slices of renal tissue of the rabbit was studied by means of the Warburg manometric method. The ischemic kidney shows definite diminution of oxygen consumption as compared with the normal kidney.

FROM AUTHORS' SUMMARY.

EFFECT OF CAROTID SINUSES ON EXPERIMENTAL HYPERTENSION. H. GOLDBLATT and others, J. Exper. Med. **71**:175, 1940.

The carotid sinus has no demonstrable influence on hypertension caused by renal ischemia, although in animals with this disease it probably plays the same part in the regulation of blood pressure as it does in normal animals.

FROM AUTHORS' SUMMARY.

EXPERIMENTAL PRODUCTION OF ENDOMETRIAL TRANSPLANTS IN THE LUNGS OF RABBITS. J. E. HOBBS and A. R. BORTNICK, Surg., Gynec. & Obst. **69**:577, 1939.

The occurrence of uterine mucosa in veins and lymphatic vessels described by Sampson, Halban and others, the well authenticated fact that syncytial cells may reach the pulmonary capillaries in pregnant women and the wide diversity of structures in which ectopic endometrium has been observed led the authors to attempt the transplantation of living endometrium into the lungs. Freshly ground portions of endometrium were injected into the ear veins of the same animals from which the tissue was obtained. Visible foci, often brownish, were produced in the lungs, and sections of these showed endometrial stroma, either alone or in combination with glands like those of the uterine mucosa, together with tissue necrosis. It is therefore concluded that endometrial transplants may grow in the lungs and that this fact offers a plausible explanation for vicarious menstruation. The aberrant tissue has characteristics which would indicate malignant potentiality.

WARREN C. HUNTER.

MAINTENANCE OF LIFE DURING EXPERIMENTAL OCCLUSION OF THE PULMONARY ARTERY, FOLLOWED BY SURVIVAL. J. H. GIBBON JR., Surg., Gynec. & Obst. **69**:602, 1939.

A method is described by which life can be maintained in an animal when the flow of blood through the heart and lungs is completely stopped by clamping the pulmonary artery. It consists of a continuous withdrawal of blood from a peripheral vein, an introduction of oxygen into the blood and a continuous return of the oxygenated blood to the animal's arterial system through a peripheral artery.

The essential features of the apparatus are a pump to withdraw the venous blood, a revolving cylinder on the sides of which the blood is oxygenated, and another pump to inject the blood back into an artery. The difference between this method and those used for perfusing isolated organs lies in the added technical difficulties entailed in the use of small peripheral vessels for the perfusion, vessels of such small size that their ligation results in no impairment of nutrition or function of the tissues supplied by them. With the use of such peripheral vessels the heart and lungs of the animal can resume their normal functions again after the removal of the clamp from the pulmonary artery and the cessation of the extracorporeal circulation. In the thirteen experiments reported the pulmonary artery was occluded for from ten to twenty-five minutes, during which time life was maintained by an extracorporeal circulation. Five animals lived twenty-four to forty-eight hours after the normal circulation was reestablished, 4 survived from eight to twenty-three days, and an equal number lived from one to nine months after a twelve to twenty minute occlusion, without neurologic or other demonstrable changes. Control experiments under identical conditions except that the extracorporeal circulation was not used demonstrated irreparable neurologic changes following occlusions of the pulmonary artery of four minutes or longer. After a ten minute occlusion life could not be restored.

From Author's Summary (Warren C. Hunter).

Anatomic and Clinical Aspects of a Demyelinizing Disease of the Monkey —Confluent Perivascular Leukoencephalomyelitis. L. van Bogaert, Ann. Inst. Pasteur **63**:315, 1939.

Intensive clinical and histologic studies were made of 11 monkeys (Macacus rhesus and four species of Cercopithecus) and 1 lemur (Maki gamböz) suffering from a spontaneous neurologic disease.

The insidious onset was sometimes accompanied by blindness, convulsive paroxysms or violent chills resembling epileptic seizures, and the later stages were characterized by paralysis. There were three clinical types, depending on the location of the nerve cells affected: (*a*) the ocular type, characterized by bilateral papillary atrophy; (*b*) the cerebral type, characterized by convulsive manifestations, simple or trembling dystonia, hemiparesis and cerebral hemiplegia or diplegia, and (*c*) the medullary type, characterized by paraplegia, first in extension and then in flexion, followed by recovery in rare cases.

These three forms are the clinical manifestations of a single histologic process involving confluent perivascular demyelinization of the white substance of the brain of a degenerative rather than of an inflammatory character.

J. B. Gunnison.

Etiology of Hodgkin's Disease. P. Uhlenhuth and K. Wurm, Ztschr. f. d. ges. exper. Med. **105**:205, 1939.

The authors did not succeed in transmitting Hodgkin's disease to animals or in developing a diagnostic serum reaction.

Pathologic Anatomy

The Amount of Lymphoid Tissue in the Human Appendix. J. M. S. Hwang and E. B. Krumbhaar, Am. J. M. Sc. **199**:75, 1940.

The amount of lymphatic tissue in the human appendix was studied in 300 persons dying violent deaths and analyzed according to eight age groups and according to sex. The percentage of the lymphatic tissue and its calculated weight were found to be the greatest in the first age group (1 to 10 years) and to diminish fairly steadily throughout life. A later secondary rise, as found in spleen material, was not observed. The appendix attains its maximum weight

in the second decade, about the age of puberty, and then decreases in weight throughout life but less markedly than does its lymphatic tissue. The female appendix apparently contains less lymphatic tissue than the male throughout life.

FROM AUTHORS' SUMMARY.

IDENTITY OF LESIONS IN B_1 AVITAMINOSIS IN PIGEONS WITH HEMORRHAGIC POLIOENCEPHALITIS IN CHRONIC ALCOHOLISM IN MAN (WERNICKE'S DISEASE). L. ALEXANDER, Am. J. Path. **16**:61, 1940.

The lesions of Wernicke's hemorrhagic polioencephalitis occurring in man and a disease produced experimentally in vitamin B_1–deficient pigeons fed ample supplies of other vitamins are identical in their topographic distribution and in their morphologic and histologic characteristics. FROM AUTHOR'S SUMMARY.

GROWTH DISORDER OF THE SKULL IN MONGOLISM. C. E. BENDA, Am. J. Path. **16**:71, 1940.

A histologic study of the synchondrosis spheno-occipitalis, of the synchondrosis sphenoethmoidalis and of tissue from the vertebrae in mongolism is presented. It is shown that proliferation of the cartilage is absent or insufficient. The histologic differences between mongolism, chondrodysplasia and cretinism are demonstrated and discussed. The growth disorder in mongolism is not restricted to the cartilaginous epiphysial lines but involves also the membranous bones. An analysis of the observations indicates that the development of the growth disorder known as mongoloid deficiency appears to be dependent on a congenital absence or deficiency of those agents from hypophysial or extrahypophysial sources which stimulate differentiation and growth. FROM AUTHOR'S SUMMARY.

HYPERTROPHY AND HYPERPLASIA OF ISLANDS OF LANGERHANS IN INFANTS BORN OF DIABETIC MOTHERS. E. B. HELWIG, Arch. Int. Med. **65**:221, 1940.

The pancreases of 9 infants of diabetic mothers and of 9 infants of nondiabetic mothers were studied. The islands of Langerhans in the infants of diabetic mothers exhibited a variable degree of hypertrophy and hyperplasia, most marked in those infants nearing full term when they were born. The nuclei were frequently enlarged and occasionally hyperchromatic. The stroma and to a less degree the islands frequently were infiltrated with eosinophilic granulocytes, many of which were mature. This infiltration was not present in the control group. One infant in the control group, whose mother was not known to have diabetes, showed hypertrophy and hyperplasia of the islands and no obvious anatomic cause of death. I am forced to conclude that some infants of diabetic mothers have definitely larger amounts of insular tissue than infants of nondiabetic mothers. This is particularly marked in those infants whose greater birth weights suggest a possible excess of growth stimulus. On the other hand, it must be recognized that a fair number of the pancreases of infants of diabetic mothers will fall in the normal range of insular size. The blood sugar levels on the whole show little correlation with insular size, the lowest level of blood sugar, 30 mg. per hundred cubic centimeters, occurring in an infant dying of subtentorial hemorrhage, and one of the higher values, 190 mg. per hundred cubic centimeters, occurring in a stillborn infant who had the largest islands of any in the series.

FROM AUTHOR'S SUMMARY.

EPILEPTOGENIC LESIONS OF THE BRAIN. W. PENFIELD and S. HUMPHREYS, Arch. Neurol. & Psychiat. **43**:240, 1940.

A meningocortical lesion with formation of a scar may result from a trauma, a healed abscess or an arterial occlusion and be the starting point for epileptiform convulsions. Excision of such a scar may stop or modify the convulsive attacks. The authors describe the histologic structure of several types of cicatrices which

resulted from trauma, inflammatory reactions, healed abscesses or meningitis, or which were caused by localized ischemia occurring during birth from occlusion of cerebral blood vessels, resulting in complete reduction in the size of a gyrus and formation of so-called focal microgyria. The meningocerebral scar is devoid of ganglion cells and for the most part also of nerve fibers. The parenchyma is replaced by a dense feltwork of fibrillary glia (termed piloid by Penfield) which is invaded by connective tissue and blood vessels. Between the scar tissue and the normal brain tissue small islands of brain substance are situated. They form a zone in which epileptic discharges seem to occur, and electric stimulation of this zone may reproduce the habitual seizure.

G. B. HASSIN.

THE CLINICAL EFFECTS OF CHRONIC EXPOSURE TO BENZENE (BENZOL). F. T. HUNTER, J. Indust. Hyg. & Toxicol. **21**:321, 1939.

Eighty-nine persons exposed to benzene fumes have been observed and studied.

Histologic material was obtained by necropsy from 8 of 10 persons who died, and by biopsy of sternal marrow from 1 person who died and in 2 who are living.

Depending on the degree and duration of exposure, an analysis of the blood pictures indicates that benzene may bring about: polycythemia or anemia; leukocytosis or leukopenia; leukemia or leukemoid blood pictures (either lymphatic or myeloid); eosinophilia; megalocytosis or microcytosis; the presence of immature marrow elements in an otherwise normal blood.

The early diagnosis of poisoning depends on an evaluation of the complete blood picture rather than on the existence of leukopenia.

Splenomegaly may appear in cases of protracted poisoning.

Women are no more susceptible to poisoning than men, and acclimatization to the fumes does not seem to occur.

The first signs of poisoning may appear with the onset of an infection, long after exposure has ceased.

It is doubtful whether any concentration of benzene greater than zero is safe over a long period of time.

FROM AUTHOR'S SUMMARY.

CHRONIC EXPOSURE TO BENZENE (BENZOL): III. THE PATHOLOGIC RESULTS. T. B. MALLORY, E. A. GALL and W. J. BRICKLEY, J. Indust. Hyg. & Toxicol. **21**:355, 1939.

Histologic material from 19 patients with a history of chronic exposure to benzene (14 autopsies and 5 biopsies) is described and analyzed. It is shown that not merely the bone marrow but the entire hemopoietic system characteristically shows changes. The picture in the bone marrow varies from severe hypoplasia to the most extreme hyperplasia and extramedullary hemopoiesis, and, in contrast to prevailing opinion, hyperplasia has proved the more common of the two reactions. It has been found only in patients with prolonged exposure, whereas hypoplasia may follow either short or long contact. Sex appears to play an important role, hyperplastic reactions being distinctly more common in males and hypoplastic ones in females. In certain of the cases of hyperplasia degrees of proliferative activity, of anaplasia and of atypicality of the hemopoietic elements not only in the marrow but in the extramedullary foci have been described which can be distinguished from neoplasia only with difficulty. Finally, 2 cases in which the pathologic pictures were typically leukemic, one showing frank tumor formation, are reported, and the cumulative evidence linking benzene with the production of leukemia is discussed.

FROM AUTHORS' SUMMARY.

RENAL LESIONS IN PURE LINE MICE. P. A. GORER, J. Path. & Bact. **50**:25, 1940.

Three types of renal disease have been found in members of three pure lines of mice (Strong's A strain, Little's C57 blacks and Strong's CBA strain) coming to postmortem examination for a variety of reasons. In mice of any strain the parietal layer of the glomerular capsule is liable to undergo metaplasia to a cubical

or columnar form. This lesion has been found most often in old members of the CBA strain. Members of the A strain are liable to have cystic or atrophic kidneys; the disease may appear at the eleventh month of age and by the eighteenth month the kidneys of almost every member of the strain are visibly diseased. A few of the C57 blacks show similar lesions, the youngest at 19 months; such lesions have not yet been observed in the CBA strain. This disease appears to be a special type of hydronephrosis; obstruction of the nephrons appears to be brought about by focal necrosis at the tips of the renal papillae. Members of the black strain aged more than 18 months frequently show lesions of the glomeruli (and occasionally of the tubules) resembling deposits of amyloid but failing to give typical staining reactions for this substance. FROM AUTHOR'S SUMMARY.

INTRANUCLEAR INCLUSIONS IN CULTURES. C. F. FISCHMANN and D. S. RUSSELL, J. Path. & Bact. **50**:53, 1940.

Acidophilic intranuclear inclusions appeared spontaneously in cultures of human fetal leptomeninges, although they were absent from the tissues from which the cultures were made. They also developed in cultures of leptomeninges from chick and rat embryos and in cultures of fibroblasts from the lungs of both human and rat fetuses. Attempts to influence the development of the inclusions by modifying the medium used for culture and by altering the environment of the experiments were unsuccessful. No evidence has been found in support of the theory that a virus is responsible for the appearance of these bodies.

FROM AUTHORS' SUMMARY.

ADAPTATION TO INCREASED INTRAVENOUS PRESSURE. PEI-LIN LI, J. Path. & Bact. **50**:121, 1940.

The normal and the pathologic histologic pictures of the portal, mesenteric and splenic veins and the inferior vena cava have been studied and the thickness of the coats of these vessels measured. In cardiac and cirrhotic diseases—conditions in which the intrahepatic pressure is presumably elevated—changes are usually found in these vessels. The changes include (1) thickening of the intima with development of longitudinal muscle fibers beneath it and (2) muscular hypertrophy in the wall of the vein. In the portal vessels the hypertrophy affects the circular muscle of the media, while in the inferior vena cava it is located in the longitudinal muscle of the adventitia. The muscular hypertrophy is believed to develop first, the intimal changes following. These changes are considered to be an adaptation to an increase in intravenous pressure, similar to the changes in arteries in hypertension. The association of changes in the portal vein and inferior vena cava is very close, but those in the latter are usually the more marked. The mesenteric vein is a direct continuation of the portal vein and frequently shows similar changes but of milder degree. The splenic vein is less frequently involved. The adaptability of the portal vessels is not influenced by age. Veins of elderly subjects can hypertrophy as much as those of young subjects. Atheromatous change has not been seen in the veins examined. It must therefore be regarded as a separate entity and of rare occurrence.

FROM AUTHOR'S SUMMARY.

CEREBRAL CHANGES IN GAUCHER'S DISEASE IN INFANCY. G. KÖHNE, Beitr. z. path. Anat. u. z. allg. Path. **102**:512, 1939.

Oberling and Woringer first described a change occurring regularly in the central nervous systems of infants with Gaucher's disease. This change, which was absent in adults presenting the disease, consisted of progressive cortical atrophy, most marked in the parietal and occipital regions and characterized by chronic Nissl cell change, chiefly in the large and medium-sized pyramidal cells. Gaucher's disease in infancy is dominated by characteristic cerebral symptoms, such as progressive muscular rigidity, hyperactive reflexes and pseudobulbar symptoms.

It was deemed desirable to further investigate the anatomic basis of these symptoms in the central nervous system. In an infant 8 months old there were found the usual microscopic changes of Gaucher's disease in the liver, spleen and other tissues. The diagnosis was confirmed by the demonstration of kerasin in the spleen. Macroscopically, the brain showed no abnormal changes. Extensive microscopic examination revealed in all regions of the gray matter of the brain a variable amount of cellular damage characterized by chronic Nissl cell change and sclerosis. The glial and rod cells were increased in number, and at times there was true neuronophagia. The ganglion cells nowhere contained any vacuoles, and Gaucher cells were not observed.

R. J. Lebowich.

Cystic Thymus in an Adult. Z. Kopaĕ, Beitr. z. path. Anat. u. z. allg. Path. **102**:560, 1939.

A rare instance of cystic thymus gland in a 36 year old woman is described. The specimen weighed 60 Gm. It occupied the anterior mediastinum, extending from the jugular fossa, above, down over the anterior wall of the heart, below. It was broken up into multiple round cysts, ranging in size up to that of a grape, which were filled with a thin clear reddish fluid. The cavities were lined by a single or multiple layer of cuboid or cylindric cells, and were separated from each other by thin septums consisting chiefly of fatty tissue and the remains of the thymic parenchyma. It was concluded that the cysts were secondary to focal necroses of the parenchyma due to vascular obstruction of unknow origin.

R. J. Lebowich.

Microbiology and Parasitology

Cultivation of the Virus of Lymphogranuloma Venereum. M. Sanders, J. Exper. Med. **71**:113, 1940.

Two strains of the virus of lymphogranuloma venereum were maintained in tissue cultures for twelve and twenty-one generations, respectively. In a third strain a quantitative increase in potency as high as one thousand times was obtained by inoculating tissue cultures with infected emulsions of known virulence. Greater increases in potency were consistently obtained by maintaining tissue cultures and virus at room temperature (23 C., with a range of approximately ± 5 C.) than by incubating them at 37 C. The virus did not survive in the absence of oxygen. Embryonic guinea pig brain and serum ultrafiltrate were found to be the most effective vehicles for the propagation of the virus. There is evidence that the site of activity of this virus is intracellular. Embryonic guinea pig brain cells were maintained in the serum ultrafiltrate, diluted with buffered salt solution, in good (morphologic) condition for as long as seventy days. Not only could old cultures be successfully inoculated with the virus of lymphogranuloma, but high titers could be maintained over extended periods.

From Author's Summary.

Localization of Bacteria in Joints and Eyes. D. M. Angevine and S. Rothbard, J. Exper. Med. **71**:129, 1940.

Histologic examination of sections of the eyes and joints of large numbers of rabbits which had received injections of hemolytic streptococci has clearly demonstrated that when arthritis or cyclitis occurs the synovial villi and the ciliary processes are the most frequent and usually the primary sites of inflammation. By special methods for the demonstration of bacteria it has been shown that bacteria which found lodgment in either an eye or a joint were demonstrable first in the vessels of the ciliary processes or of the synovial villi. Localized synovitis or iridocyclitis is brought about by the localization of bacteria in the synovial villus or in the ciliary process. These experiments, which give a clearer insight into the genesis of infectious arthritis and iritis, explain why both may occur in association with certain infectious diseases.

From Authors' Summary.

STRAIN OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS. J. CASALS-ARIET and L. T. WEBSTER, J. Exper. Med. **71**:147, 1940.

A strain of the virus of lymphocytic choriomeningitis has been encountered which grows readily in mouse embryo–serum–Tyrode culture medium. Its origin is not definitely known but appears to be either the mouse brain tissue or, more probably, the monkey serum. This strain gives clearcut results on filtration tests through Elford membranes, establishing the size of the virus, according to formula, as 33 to 50 millimicrons. The strain shows a high uniform virulence in W-Swiss mice. This appears to be due in part, at least, to the age and strain of mice employed for passage and titration. The strain has been found to be more virulent in young than in old mice, especially following intraperitoneal inoculation. Finally, this strain when given as a vaccine intraperitoneally in amounts as small as 160 intracerebral lethal doses induces immunity against subsequent intracerebral inoculations of as much as 10,000 lethal doses. FROM AUTHORS' SUMMARY.

SYMPTOMATIC HERPES, SEQUELA OF INDUCED FEVER. S. L. WARREN, C. M. CARPENTER and R. A. BOAK, J. Exper. Med. **71**:155, 1940.

Symptomatic herpes occurred in 190 (46.2 per cent) of 411 patients treated with fever induced by physical methods. Herpes recurred in only 7 (5.3 per cent) of 131 patients given subsequent fever treatments, suggesting that some immunity develops with the first attack. An acute encephalitis-like syndrome of short duration and without sequelae developed in a group of patients with severe herpes following fever therapy. A filter-passing virus isolated from herpetic vesicles on patients treated with artificially induced fever produced fatal encephalitis in rabbits when inoculated intracerebrally and by corneal scarification. Intranuclear inclusion bodies were observed in corneal epithelial cells and in motor ganglion cells of the brain similar to those observed in rabbits inoculated with known strains of herpes virus. Four strains of virus that had been recovered from herpetic vesicles appearing on patients subsequent to artificially induced fever were shown by cross protection tests on rabbits to be immunologically related to the Frank strain of herpes virus. FROM AUTHORS' SUMMARY.

TOXOPLASMIC ENCEPHALOMYELITIS. A. WOLF, D. COWEN and B. H. PAIGE, J. Exper. Med. **71**:187, 1940.

Infected material from the brain and spinal cord of an infant suffering from a recently recognized form of granulomatous encephalomyelitis was inoculated into rabbits and mice and produced an experimental disease which was readily transmissible in series. A parasite identical with that in the lesions of the infant was found in the lesions of the experimental animals. The morphologic characteristics of this micro-organism, the course of the disease, the lesions produced in the animals inoculated with it, the wide host range of the parasite and the results of cross immunity experiments establish its identity as a Toxoplasma. It is suggested that the micro-organism be designated Toxoplasma hominis. The clinical and pathologic features of the infection produced in animals by this parasite are described. The infection in the infant is the first proved instance of human toxoplasmosis. Since the lesions were confined to the central nervous system, the disease is termed toxoplasmic encephalomyelitis. The first experimental transmission of human toxoplasmosis to animals is recorded.

FROM AUTHORS' SUMMARY.

SEPARATION OF MIXTURES OF BACTERIAL SPECIES. J. H. HANKS, R. B. HOULIHAN and L. W. PARR, J. Infect. Dis. **66**:33, 1940.

In a mixture of bacterial species, serum sensitization of the predominating organisms was found to promote greatly the isolation of the unsensitized bacterial species.

This procedure was compared with other means which influenced the bacterial ratios in a favorable manner. So far as typhoid bacilli were concerned, capillary migration and differential resuspension in the presence or absence of sensitizing antibody were effective means of reducing the interference by colon bacillus colonies.

FROM AUTHOR'S SUMMARY.

ISOLATION OF BRUCELLA FROM LYMPH NODES. M. A. POSTON and P. B. PARSONS, J. Infect. Dis. **66**:86, 1940.

A method used successfully for the isolation of Brucella from lymph nodes is described in detail.

Lymph nodes from 10 of 19 patients with Hodgkin's disease have yielded positive cultures of Brucella. Seven of the positive cultures were of the suis variety and 3 were of the melitensis variety.

Cultures of lymph nodes from 3 patients with chronic brucellosis without clinical evidence of Hodgkin's disease were positive for Brucella melitensis var. melitensis in 2 instances and Brucella melitensis var. suis in 1 instance.

In a control series of cultures from 67 lymph nodes which were taken for biopsy because of a variety of conditions only 1 culture showed Brucella melitensis var. suis.

FROM AUTHORS' SUMMARY.

EPITUBERCULOSIS. W. B. WOOD, Tubercle **20**:205, 1939.

Epituberculosis (Eliasberg and Neuland, 1920) is a manifestation of postprimary tuberculosis in childhood and occurs in a period characterized by enlargement of the bronchial glands and a tendency to exudation. The chief factor in its production is atelectasis due to blocking of bronchi by tuberculous glands. A secondary factor is probably exudation into the alveoli and engorgement, the result of lymph stasis and vascular congestion. Tuberculous allergy possibly also plays a part, for allergic reactions are common in the second stage. If caseation of a gland leads to rupture of the wall of a bronchus, caseous bronchitis or bronchopneumonia may entirely alter the clinical picture and cause permanent damage to the lung. If the bronchial wall escapes such ulceration, restitution can occur and the collapsed lobe return to its original condition. A failure to reexpand must in either event give rise to bronchiectasis, which is not an uncommon result of tuberculous adenitis. One cause of the hilar flare phenomenon is atelectasis of the middle lobe of the right lung due to bronchial compression. Collapse of the apical division of the lower lobe may cause a similar shadow and have a comparable origin. Many of the triangular shadows based on the hilus are evidently due to local atelectasis, but care is needed in interpretation because other lesions of a tuberculous and of a nontuberculous nature may cause similar opacities. The term "epituberculosis," which has served its purpose and drawn attention to the condition, might now be abandoned.

H. J. CORPER.

EFFECTS OF VARYING DEGREES OF ALLERGY IN EXPERIMENTAL TUBERCULOSIS. C. CLAYSON, Tubercle **20**:533, 1939.

A very marked degree of allergy is dangerous in the event of a massive superinfection, but the significance of mild and marked allergic states has been finally elucidated. The relationship of the phenomenon of Koch to the development of allergy is not clear. A marked degree of allergy may exist without the development of the true phenomenon of Koch, and conversely the true phenomenon of Koch can take place in the presence of a slight degree of allergy. The formation of pus in glandular lesions appears to be an allergic phenomenon. The extent of visceral disease was not shown to bear any relationship to the degree of allergy at superinfection. Serous effusions were not significantly commoner among markedly or very markedly allergic animals subjected to superinfection than among feebly allergic animals similarly superinfected or among nonallergic animals.

H. J. CORPER.

Immunology

FIBROSARCOMA AS A RESULT OF THE INJECTION OF RADIUM CHLORIDE. F. NØRGAARD, Am. J. Cancer **37**:329, 1939.

A report is given of a case of tibial fibrosarcoma produced by the injection of 10 micrograms (0.000,01 Gm.) of radium chloride into the knee joint eight or nine years previously on account of arthritis. It was demonstrated that the patient was radioactive and that in addition to the sarcoma there were hyperplasia of the bone marrow and radiation osteitis, indicating radium poisoning. The femur was amputated, and the bones of the knee joint were examined for the amount of radium salt remaining in them. The case is discussed with reference to the few existing clinical observations and some experimental investigations. The conclusion is drawn that the administration, whether oral or parenteral, of even minimal quantities of radioactive substances to the organism will in all probability lead to the development of malignant tumors. Such administration ought—practically speaking—never to take place either for therapeutic or for diagnostic purposes.

FROM AUTHOR'S SUMMARY.

BROWN DEGENERATION OF ADRENALS AND MAMMARY CANCER IN MICE. W. CRAMER and E. S. HORNING, Am. J. Cancer **37**:343, 1939.

The present conception of the significance of the degeneration in the adrenal gland for the etiology of mammary cancer may be summarized as follows: It is not in itself the cause of mammary cancer, since in the high cancer strains it develops in practically every mouse, male or female, whether the mammary glands of these animals are cancerous or not. Nor is the development of cancer in the mamma absolutely dependent on its presence, since in the low cancer Bagg Albino strain and in the mixed strains mammary cancer may develop while the adrenal is intact. The factors which determine the onset of cancer—what the authors call the remote causes of cancer—are as varied for the mamma as they are for the skin and probably for most other organs or tissues. One of these remote causes is an endocrine imbalance, and the spontaneous development of degeneration in the adrenal medulla is one of the ways in which such an endocrine imbalance is brought about.

FROM AUTHORS' SUMMARY.

LATENT PERIOD OF LYMPHOMATOSIS IN DILUTE BROWN MICE. G. B. MIDER and J. J. MORTON, Am. J. Cancer **37**:355, 1939.

Forty-eight of 60 dilute brown mice that were painted with methylcholanthrene in benzene acquired leukoses. Four types of reaction are described: general lymphomatosis, localized mediastinal lymphoma, extramedullary myelopoiesis, reticuloendotheliosis. Spontaneous lymphomatosis occurred in members of the strain that lived six hundred and fifty to eight hundred days. Painting with methylcholanthrene reduces the latent period of lymphomatosis by more than one half. A comparable effect is not obtained by painting with benzene alone.

FROM AUTHORS' SUMMARY.

EXPERIMENTAL PRODUCTION OF BRAIN TUMORS IN MICE WITH METHYLCHOLANTHRENE. A. M. SELIGMAN, M. J. SHEAR and L. ALEXANDER, Am. J. Cancer **37**:364, 1939.

Tumors have been produced in the brains of male C_3H mice in eight to seventeen months by the intracerebral implantation of pellets of 20-methylcholanthrene. Of the 20 mice employed, 11 acquired gliomas, and 2, fibrosarcomas, which were probably of meningeal origin. The gliomas were of several types; some closely resembled characteristic subtypes of human glioma. One glioma and one fibrosarcoma were successfully transplanted subcutaneously in other mice of the same

strain; these tumors are now in their fifth and eleventh generations, respectively. Intracisternal injection of methylcholanthrene in lard solution in strain D mice and as crystals in rats and in strain A and strain D mice failed to produce tumors. Methylcholanthrene pellets introduced subcutaneously in other male C_3H mice induced sarcomas around the pellets. These sarcomas appeared more rapidly than did the induced brain tumors.

FROM AUTHORS' SUMMARY.

MOLECULAR WEIGHT OF DIPHTHERIA TOXIN AND ANTITOXIN. A. M. PAPPENHEIMER JR., H. P. LUNDGREN and J. W. WILLIAMS, J. Exper. Med. **71**:247, 1940.

Purified diphtheria antitoxic horse pseudoglobulin has been prepared which is homogeneous by sedimentation, diffusion and electrophoresis. Immunologically, however, the preparation contains only 43.5 per cent antitoxin specifically precipitable by toxin. The inactive pseudoglobulin remaining after specific precipitation was found to have the same physical and chemical properties as the original antitoxic pseudoglobulin. Although the molecular weight of the antitoxin is the same as that of the normal horse serum globulins, the electrophoretic mobility differs from those normally present. The molecular weight of diphtheria toxin is 70,000 and that of antitoxin is 150,000. From ultracentrifuge studies on the two reactants and on mixtures of toxin and antitoxin in the soluble inhibition zones, the average molecular composition of the specific floccules at certain reference points throughout the equivalence zone and the maximum "valence" of toxin and antitoxin with respect to each other have been calculated. The significance of the results has been discussed in relation to antigen-antibody reactions in general, and a possible explanation for the exceptional behavior of the toxin-antitoxin reaction in the region of excess antitoxin has been suggested.

FROM AUTHORS' SUMMARY.

ANTI-EGG ALBUMIN ANTIBODY IN HORSE. A. M. PAPPENHEIMER JR., J. Exper. Med. **71**:263, 1940.

The reaction between three times recrystallized egg albumin and its antibody in the horse has been studied. The reaction exhibits a behavior typical of the Ramon diphtheria flocculation reaction.

FROM AUTHOR'S SUMMARY.

A QUANTITATIVE THEORY OF THE PRECIPITIN REACTION. H. HEIDELBERGER, H. P. TREFFERS and M. MAYER, J. Exper. Med. **71**:271, 1940.

Of 2 rabbits subjected to a prolonged course of injections of crystalline egg albumin, the antibodies in one showed progressive changes such as were noted in an earlier paper; the antibodies in the other did not. The significance of this behavior in the production of serum for therapeutic use is pointed out. Quantitative studies are reported on the low grade or incomplete antibody present in the early stages of immunization of a horse with egg albumin. Quantitative studies on the flocculating antibody from later bleedings from the horse are given, and the dissociation of the soluble prezone compounds by rabbit antibody for egg albumin is studied. Rough estimations of velocity are reported. The bearing of the findings on the mechanisms of precipitin and flocculation reactions and of the Danysz effect is discussed in terms of the union of multivalent antigen with multivalent antibody.

FROM AUTHORS' SUMMARY.

FORMATION IN SKIN OF ANTIVACCINIAL ANTIBODIES. G. HARTLEY JR., J. Infect. Dis. **66**:44, 1940.

Intradermal inoculations of aluminum hydroxide gel cause a mobilization of cells consisting almost entirely of macrophages, which are capable of phagocytosing the gel.

When small quantities of vaccinia virus (testicular or neural strains) are adsorbed to aluminum hydroxide gel and are inoculated into tissues in which large numbers of macrophages are present, neutralizing antibodies are often demonstrable in extracts of this tissue before they are present in the undiluted blood serum or in other tissues.

Inasmuch as this tissue consists predominantly of macrophages capable of ingesting aluminum hydroxide gel to which the virus has been adsorbed, the conclusion seems warranted that these neutralizing antibodies are produced by the macrophages in this area.

FROM AUTHOR'S SUMMARY.

BLOOD GROUPS IN AFRICA. R. ELSDON-DEW, Publ. South. African Inst. M. Research **9**:29, 1939.

The results of a blood-grouping expedition to East Africa are given, which together with the author's previous results makes a total of some twenty thousand. Forty-six tribes were examined, and the results are compared and contrasted with forty-three observations by other authors.

A cartesian type of reasoning has been applied to the results, and the author postulates a mutation of B in the African distinct from that occurring in the Hindu. It is probable that this mutation occurred in localized areas, the peoples concerned being the Pygmies and the Egyptians.

Further proof is adduced that the Bantu did not descend from the Negro but are a purer example of the common stock. The migration on the west coast has been from east to west rather than in the contrary direction.

The Bush people are shown to be less "ancient" than are the Bantu, and an Egyptian origin is suggested for the Hottentot.

Certain fundamental principles in the interpretation of ethnologic blood groupings are outlined in the text.

FROM AUTHOR'S SUMMARY.

Tumors

ARTIFICIAL BENIGNANCY OF NEOPLASM. J. MUUS, F. N. CRAIG and W. T. SALTER, Am. J. Cancer **37**:553, 1939.

Pedigreed albino mice, Bagg strain A, were "immunized" against sarcoma 180 and later inoculated with malignant sarcoma tissue from a single tumor. From this material subcutaneous sarcomas were selected, forming a graded series, varying from tiny "benign" growths to large "malignant" ones. Although these tumors are known to vary seriatim in their respective mitotic activities, no clear difference in lactic acid or ammonia metabolism could be established by biochemical studies. Oxygen consumption, however, was low in "benign" tumors.

FROM AUTHORS' SUMMARY.

RETENTION OF IMMUNIZING POWER BY THE SHOPE PAPILLOMA AFTER EXPOSURE TO ULTRAVIOLET RADIATION. W. H. WOGLOM and J. WARREN, Am. J. Cancer **37**:562, 1939.

Of 12 rabbits given intraperitoneal injections of an ultraviolet-irradiated extract of the Shope papilloma, 11 proved totally immune to subsequent inoculation with the virus of this growth and 1 virtually so, while all 7 untreated controls acquired tumors.

FROM AUTHORS' SUMMARY.

THE FUCHS REACTION FOR CANCER. O. ROSENTHAL, Am. J. Cancer **37**:566, 1939.

It is pointed out that, of all the cancer tests which are based on reactions between serum and cancer-specific substances, only the test of Fuchs has been verified by many authors. Since some technical difficulties to which occasional failures of the reaction had been ascribed were eliminated recently by Minibeck,

Rosenthal decided to study the diagnostic and prognostic value of the test by means of Minibeck's technic. It was found that the reaction of Fuchs demonstrates differences between cancer and noncancer serum only in a statistical sense; it is without real value for dignosis in an individual case. The chief difficulty of the test lies neither in the technic of the reaction nor in the preparation of the substrates but in the fact that the selection of specific substrates of such activity that the reaction values are unquestionably beyond the range of error is a matter of chance. A comparison of the results of other authors using the Fuchs test supports these observations, although the conclusions of most of these authors are more favorable. Whether the test can be of any aid in the diagnosis and prognosis of cancer in the patient can be decided only if the mechanism of the reaction can be explained in detail. It is possible that it can be still further elucidated by animal experimentation.

FROM AUTHOR'S SUMMARY.

POLAROGRAPHIC STUDIES OF HUMAN BLOOD SERUM. A. C. WALKER and S. P. REIMANN, Am. J. Cancer **37**:585, 1939.

The effect of pathologic conditions on the sulfur-containing groups in human blood serum has been studied by means of the polarographic method of chemical analysis. The samples used were from 386 patients, including approximately 100 each of normal subjects and cancerous ones, the remainder being patients with miscellaneous pathologic conditions. The method is shown to be remarkably reproducible and sensitive for measuring the content of these sulfur groups, which appear to be in the form of a cysteine-cystine equilibrium. The majority of serums from cancerous patients give values outside the distribution curve for normal serum. The larger the deposits of cancer the more consistent is the difference from the normal. Such factors as temperature, age, kind of tumor and general bodily health influence the cysteine-cystine equilibrium. Since sufficient data on these points have not been secured as yet, the method cannot be used as a means of diagnosing cancer at the present time. FROM AUTHORS' SUMMARY.

DIFFUSE MENINGIOMATOSIS. L. M. WEINBERGER, Am. J. Cancer **38**:1, 1940.

A meningioma is reported which existed as a thin crust extending over the surface of the larger portion of the left frontal and temporal lobes. An analysis of this tumor and the few similar ones recorded in the literature shows that three different types of diffuse benign mesodermal tumor can be identified: a type in which neither of the leptomeningeal layers can be distinguished, both being incorporated in the tumor; a type in which the pia is normal but the arachnoid is widely converted into tumor; a type in which the arachnoid is normal but the pia is the site of diffuse tumor formation. Close examination indicates that these tumors covering large areas of the brain arise in situ. Their peculiar conformation cannot be explained by implantation of tumor cells from a distant source or by eccentric growth and permeation from a germinative focus. They are benign, according to acknowledged criteria.

FROM AUTHOR'S SUMMARY.

OBSERVATIONS ON ANIMALS PAINTED WITH TOBACCO TAR. K. SUGIURA, Am. J. Cancer **38**:41, 1940.

Prolonged painting of the skin of mice with combustion products of tobacco distilled over at temperatures between 100 and 900 C. produced a single instance of squamous carcinoma among 168 Bagg albino mice that lived from ninety to five hundred days. Painting the skin of C57 black mice and dba mice, as well as the ears of rats of Wistar Institute stock and of rabbits of common stock, with tobacco tar failed to produce any cancerous change. The proliferating capacity of mouse sarcoma 180 and of the Flexner-Jobling rat carcinoma was unaffected by tobacco tar or pure nicotine solution when the solutions were adjusted to p_H 7.4.

FROM AUTHOR'S SUMMARY.

ORIGIN OF PERINEURAL FIBROBLASTOMA. I. M. TARLOV, Am. J. Path. **16**:33, 1940.

Direct evidence obtained by a staining technic that brings out normal and proliferating Schwann cells excludes the Schwann cell as the type cell of the common encapsulated tumors of peripheral nerves. The type cell of these tumors presents the morphologic characteristics of the fibroblast. The term "perineural fibroblastoma" should be retained for these tumors, although it must be realized that they may arise from endoneurium, as in the case of the tumors of the vagus nerve roots which are reported in this article. Although the occurrence of nerve fibers within an encapsulated tumor of the peripheral nerves is usually characteristic of multiple neurofibromatosis, an occasional nerve fiber may occur within the solitary perineural fibroblastoma.

FROM AUTHOR'S SUMMARY.

SCHWANN CELL VERSUS FIBROBLAST AS THE ORIGIN OF THE SPECIFIC NERVE SHEATH TUMOR. M. R. MURRAY and A. P. STOUT, Am. J. Path. **16**:41, 1940.

Murray and Stout offer evidence, obtained by means of tissue culture, that the specific nerve sheath tumor, or neurilemmoma, has a Schwann cell origin. This evidence is based on morphologic and physiologic similarities between the schwannian outgrowth from normal and experimental nerves and the outgrowth from spontaneous tumors. It is inferred that Schwann cells can and do condition the formation of collagen without the intervention of fibroblasts, since these tumors, whose outgrowth is wholly schwannian, contain considerable collagen. The tumor cells' behavior in vitro offers an explanation of the so-called cystic degeneration in these tumors, which harmonizes this degeneration with the sclerosis seen in neurofibromas.

FROM AUTHORS' SUMMARY.

THE AGENT PRODUCING LEUKOSIS AND SARCOMA OF FOWLS. E. A. KABAT and J. FURTH, J. Exper. Med. **71**:55, 1940.

The activity of the sarcoma or leukosis producing agent in material deposited by high speed centrifugation is the same as that of the agent in the original crude extracts. Material sedimentable at high speed containing approximately 10^{-5} mg. of nitrogen produces tumors at the sites of injection. Small quantities of material sedimentable at high speed are present in normal chicken serum, and about twice as much in leukemic serum (strain 1). Normal chicken and mouse spleens and all other human and mouse tissues examined contain large amounts of material sedimentable at high speed. Extraction of leukemic blood cells with saline solution yields little additional virus. The washed cells readily produce leukosis even after irradiation with amounts of roentgen rays sufficient to destroy the leukemic cells but not the virus. Freezing at —60 C. preserves the activity of the high speed deposits for at least six months. Addition of 5 per cent of saturated sodium sulfate solution slightly delays deterioration of high speed deposits in the ice box. Most of the agent measured by inoculation of chickens and the fraction sedimentable at high speed measured by its nitrogen content are precipitated by one-third saturation with sodium sulfate.

FROM AUTHORS' SUMMARY.

ACTIVE AGENT OF THE ROUS SARCOMA NO. 1. A. POLLARD, Brit. J. Exper. Path. **20**:429, 1939.

Pollard has undertaken the preparation of pure suspensions of the tumor-producing agent of Rous sarcoma 1 by methods of centrifugation and has investigated their chemical composition. The preparations contained an inactive lipoid fraction and an active protein fraction containing both phosphorus and carbohydrates, probably as nucleic acid. Products derived by centrifugation from nonfiltrable tumors were found to be similar in general composition to the Rous agent, although they possessed no infectivity. The picture of such products and their occurrence in the malignant cell are discussed. It is considered that the Rous agent, although consisting mainly of nucleoprotein, may be more complex in character than is bacteriophage, which has been found to consist almost entirely of nucleoprotein.

Society Transactions

NEW ENGLAND PATHOLOGICAL SOCIETY

SIDNEY FARBER, *President*

BENJAMIN CASTLEMAN, *Secretary*

Regular Meeting, Feb. 15, 1940

Pathologic Observations in Simmonds' Disease. RICHARD C. WADSWORTH.

A case of pituitary cachexia is presented because of the unusual changes in the pituitary gland, which may have some bearing on the genesis of at least one type of Simmonds' disease. Although it has been shown that Simmonds' disease is due in certain cases to gumma, tumor or tuberculosis, in most of the cases described in the literature the disease was considered to have developed as a result of thrombosis or embolism of the arteries of the pituitary gland.

A 51 year old white married woman, 4 feet 8 inches (142 cm.) tall, was admitted to a hospital for patients with mental disease because of symptoms suggesting a manic-depressive psychosis. She married at the age of 37, and one year later she gave birth to a child. High forceps were used and the postpartum course was characterized by twelve days of fever, the temperature reaching 103 F. Following delivery, the patient lost weight and failed to recover strength. The mental symptoms first appeared during the postpartum period. The blood and spinal fluid were normal. There was gradual loss of axillary and pubic hair. The breasts became atrophic and the skin thick, dry and scaly. The weight dropped to 81 pounds (36.5 Kg.); the basal metabolic rate, to —36 per cent. One morning the patient was found unconscious, with a poor pulse, bilateral extensor rigidity of the arms and legs, bilateral foot clonus and a Babinski sign on the right. There was marked hypoglycemia with blood in the spinal fluid. The total protein in the spinal fluid was 127 mg. per hundred cubic centimeters.

Autopsy revealed fibrosis of the anterior lobe of the pituitary gland with evidence of a progressive destructive nonspecific granulomatous process, manifested by *unique subendothelial fibrinoid nodules in the venous sinuses,* lymphocytic infiltration and numerous old and recent hemorrhages. There was atrophy of the thyroid, adrenal and parathyroid glands and of the ovaries, with microsplanchnia and scattered hemorrhages throughout the nervous system. There were also acute enteritis and bronchopneumonia.

DISCUSSION

CHARLES H. LAWRENCE (by invitation): This syndrome of marked loss of weight or loss of personality and various functional disturbances is the subject of a great deal of discussion. If the patient is seen first by a psychiatrist, a diagnosis of anorexia nervosa is made, and if the patient is seen by an enthusiastic endocrinologist, the condition is labeled Simmonds' disease. It seems to me that there are certain clinical features which should prevent one from going wrong very often in the diagnosis of this syndrome. In the first place, in definite Simmonds' disease there is almost always evidence of failure of the other glands which are under the influence of the anterior lobe of the pituitary gland, such as a disturbance of the menstrual cycle, loss of libido and loss of pubic hair. It also seems to me that in true anorexia nervosa the change in personality is much more definite and convincing than it is in the patients with changes in the pituitary gland.

B. EARLE CLARK: Are there any more details concerning the obstetric history, especially as regards considerable loss of blood or anything resembling shock?

Sheehan of Glasgow has described postpartum necrosis of the anterior lobe of the pituitary gland in patients who die soon after delivery. He believes that many of the patients with Simmonds' disease are those who recover from this necrosis.

LEO ALEXANDER: Did you observe any similar vascular proliferations in any of the other organs or in any other material?

RICHARD C. WADSWORTH: So far I have not seen lesions just like this in other material. Some of the subendothelial fibrinoid nodules suggest somewhat the lesions of rheumatic fever, but I was unable to find any evidence of rheumatic fever in this case.

Mechanism of Sensitization to Organic Anhydrides. JOHN L. JACOBS (by invitation).

By a recent method (Jacobs, J. L.; Golden, T. S., and Kelley, J. J.: *Proc. Soc. Exper. Biol. & Med.* **43**:74, 1940) guinea pigs were sensitized to citraconic anhydride and irregularly to phthalic anhydride so that wheal and erythema reactions immediately followed scratch tests with the homologous substances. Cross tests with citraconic and phthalic anhydrides (both in dioxane [diethylene dioxide] solution) in animals sensitized to one or the other of the two substances demonstrated conclusively that these immediate reactions in scratch tests were specific phenomena. Furthermore, of 18 guinea pigs sensitized to citraconic anhydride, 3 showed an immediate general reaction, a pinkish erythema over almost the entire skin, appearing first about ten minutes after the scratch test was made and lasting for several hours. The skin appeared to be somewhat thickened, and the reaction was strongest on the flank tested and at old sites of injection. All reaction except in the vicinity of the scratch had entirely disappeared by the following day. The possibility that citraconic anhydride or a conjugate of some sort spreads through the body with great rapidity is being studied. The general reaction resembles phenomena observed in man after the administration of various drugs.

Attempts were made to transfer hypersensitivity to these substances by the Prausnitz-Küstner technic, with evident success in preliminary experiments. In the experiments, however, the substances used for testing were complex antigens, prepared by combining citraconic anhydride and phthalic chloride, respectively, with guinea pig serum. Sensitizing injections of 0.2 cc. of serum from a sensitized animal were made intracutaneously in a normal pig, and after three days 0.02 cc. of a 0.1 per cent solution of the test antigen was injected at the same point. Specific immediate wheal and erythema type reactions were observed, reaching a maximum in about thirty minutes and diminishing thereafter. This experiment indicates a participation of humoral antibodies in hypersensitiveness to chemicals of this type.

DISCUSSION

ABRAHAM COLMES (by invitation): The sensitizing of guinea pigs to chemicals was well conceived by Dr. Jacobs and well carried out. I had the privilege of seeing some of the animals, and the immediate reaction to a simple scratch test was impressive. The problem is one of clinical sensitivity to a drug—a type which physicians see rather often and which is difficult to reproduce in animals of the lower type. Physicians know that each drug tends to produce a definite type of symptoms: aspirin (acetylsalicylic acid) may induce asthma and urticaria; aminopyrine seldom produces cutaneous lesions but does produce agranulocytosis; the mercury and iodide groups produce characteristic changes in the skin. Drugs are to be contrasted with protein antigens, all of which are capable of producing similar reactions. Ragweed and house dust, for example, can sensitize human beings, with the development of hay fever and bronchial asthma, respectively. It is not to be wondered that the anhydride group of chemicals have their own characteristics, and sensitization with immediate cutaneous reaction is one of them. At the suggestion of Dr. Jacobs my associates and I have tried to reproduce sensitivity to the anhydride group in human beings. We have succeeded in injecting into the skin of several persons 1 cc. of a 1:1,000 dilution of citraconic anhydride in olive oil. The immediate reaction after the first injection was not different from

that of the control site in which there was an injection of olive oil alone. I followed one of the treated patients for some time. Forty-eight hours after the first dose I noted redness and induration at the site of inoculation. This persisted and increased in intensity. Evidently, citraconic anhydrine in olive oil tends to be retained at the site of injection and eventually hooks up with some protein of the body, forming a conjugate protein with a specificity of its own. This probably constitutes the mechanism involved in citraconic anhydride sensitization. Incidentally, the patient mentioned was followed because of asthma, and he told me that each time he had an attack of asthma the skin lesions from sensitization with citraconic anhydride became reactivated. This brings to mind the reactivation of specifically sensitized tissues by nonspecific stimulants, so often noted in clinical allergy of the atopic type. It suggests that all allergies have a common basis, differing chiefly with the species involved and with the substances used in sensitization.

ETHAN ALLAN BROWN (by invitation): It seems to me that this paper brings up more questions than it answers. It is worth remembering that there are chemicals, such as acetylsalicylic acid and phenobarbital, which do not give wheals when the patient's skin is tested with them and yet when ingested cause urticaria in sensitive subjects. Another group of urticariogenic substances is evidently released by bacteria and food toxins. In another field, dermatitis due to contact sensitivity is due to oil-soluble proteins. This type of sensitivity is not associated with a wheal but with a special eczematoid type of reaction. I bring up these various facts because they are partially related to the subject under discussion. Dr. Jacobs' work seems to show that the guinea pig sensitized to citraconic anhydrine or to phthalic anhydride gives a wheal and erythema. Cross tests showed that these reactions to the scratch tests were a specific immunologic phenomenon. Superficially, it seems that the technic followed should give a contact type of dermatitis rather than a reaction of the wheal type. A knowledge of the properties of phthalic anhydride may give the answer. Dr. Jacobs, of course, knows this but did not mention that phthalic anhydride is both oil and water soluble. The problem then can be postulated in this form: Is it that a simple chemical substance when applied with the present technic gives a true allergic response, as shown by a wheal; or is it really that the same substance, being both oil and water soluble, has acted in two ways? First it has been absorbed as a contactant; second, once in the system it has acted as a water-soluble protein. Further work would probably show that this was true.

Pathologic Observations on Leukoerythroblastic Anemia. S. B. THORSON.

In 1936 Janet Vaughan defined leukoerythroblastic anemia as a type of anemia in which immature red cells and a few immature white cells of the myeloid series appear in the peripheral circulation. She described 13 cases of this type of anemia associated with (1) carcinoma with metastases to the marrow, (2) myelomatosis, (3) myelosclerosis and (4) Cooley's erythroblastic anemia. Hickling, in 1937, described 7 cases of a similar blood picture under the title of "chronic non-leukemic myelosis," in 5 of which there was no involvement of bone marrow.

In June 1932 a 31 year old white woman suffered from "acute polyarticular arthritis" following appendectomy and ventral suspension of the uterus. With salicylate therapy there was recovery within eight days. In January 1933 she complained of menorrhagia and purpuric spots. Blood platelets were absent, and the bleeding time was prolonged. A diagnosis of "idiopathic thrombopenic purpura" was made. In a week she was discharged improved, without treatment. During the next five years she continued to have purpura and menorrhagia with a gradual enlargement of the spleen, shortness of breath, faintness and palpitation. There was progressive severe anemia, which did not respond to iron or liver. When examined by Dr. W. B. Castle in February 1938 she presented a slightly icteric pallor, a palpable spleen, severe leukoerythroblastic anemia, mild leukopenia, slight thrombopenia, reticulocytosis, an elevated icterus index, an indirect van den Bergh reaction and increased fragility of the red blood cells. A diagnosis of "chronic

nonleukemic myelosis" was made. The red cell count dropped to 780,000 and the hemoglobin (Sahli) to 18 per cent. There was a temporary response to three blood transfusions. The patient died March 2.

Autopsy.—There were terminal bronchopneumonia and multiple pulmonary embolism, restricted to the smaller vessels. The marrow was uniformly red and extremely cellular. The reaction was essentially erythroblastic, with islands of primitive erythroblasts surrounded by large numbers of maturing red cells. Granulopoiesis was slight. The spleen showed an extensive venous infarction. The veins of the pulp contained fresh thrombi. Small islands of erythropoiesis were present. The liver showed small areas of erythropoiesis. The lymph nodes of the abdomen revealed erythropoiesis and slight erythrophagocytosis and hemosiderosis.

Vaughan emphasized the bone lesions of this disease. Hickling noted that only 2 of his 7 patients had involvement of bones. He also has stressed the close similarity of this disease clinically to "aleukemic myelosis." The lesions in the bones appear to be late manifestations. Had my patient not succumbed to a hemolytic crisis, myelosclerosis might well have been the end result. The etiologic factors are unknown.

DISCUSSION

WILLIAM DAMESHEK (by invitation): To my mind the condition described by Dr. Thorson is an example of acute or subacute hemolytic anemia, which is the same as acquired hemolytic jaundice of an acute or subacute type. Some years ago this person had thrombopenic purpura. Superficially thrombopenic purpura and hemolytic jaundice do not have anything in common, but fundamentally they may be related. Thrombopenic purpura, although a condition of the marrow due apparently to a failure of megakaryocytes to produce platelets, may more fundamentally be due to a disorder of the spleen that inhibits the formation of megakaryocytes in the marrow. The thrombopenic purpura in this case might have been due to a splenic condition. Chiefly because of the dramatic effects of splenectomy, my colleagues and I believe that hemolytic jaundice has at least something to do with the spleen. It is conceivable that the spleen might at one time have produced thrombopenic purpura and at another time hemolytic jaundice. This is highly speculative, to be sure, but is thrown out as a suggestion.

The outstanding feature of this case is not only anemia but also acholuric icterus, which immediately suggests the possibility of hemolytic anemia. Furthermore, the high reticulocyte count, present at all times, is highly indicative of the regenerative tendencies which always come into play in a hemolytic process. The blood picture corresponds closely to what has been described recently as "macrocytic hemolytic anemia" and with what I have designated as "pseudomacrocytosis" in acute or subacute hemolytic anemia. I believe, therefore, that this was a case of acquired hemolytic jaundice, the nucleated red cells, myelocytes and other abnormal findings in the blood all being indicative of pronounced regenerative efforts on the part of the marrow. I believe, too, that splenectomy might well have been indicated.

WILLIAM B. CASTLE (by invitation): This case presents to my mind features of two conditions or possibly a terminal acceleration of a single process. In the article on "non-leukemic myelosis" referred to by Dr. Thorson, Hickling described certain features exemplified in this patient. First, the long duration—years; second, the large spleen, and, third, the purpura of thrombopenic type in the past.

The final blood picture in Dr. Thorson's case presents, first, mild leukopenia with myeloid elements that were immature but not immature enough to allow a diagnosis of leukemia, and thrombopenia; second, an increased percentage of reticulocytes, icterus and a slight increase in the fragility of the red blood cells. Therefore, one must infer that there was a hemolytic process. This, however, had apparently begun only within weeks or months and was not observed with the earlier episode of purpura. As Dr. Dameshek has said, it would therefore be proper to state that the patient had an acquired hemolytic jaundice. However, in my experience, in patients with hemolytic anemia—except those with the curious

disease originally described by Marchiafava (paroxysmal nocturnal hemoglobinuria) and recently analyzed by Dr. Thomas H. Ham at the Boston City Hospital—one finds leukocytosis and an abundance of platelets. Thus, the blood picture in Dr. Thorson's case is not typical of the usual hemolytic anemia, because of the leukopenia and thrombopenia. If one regards the entire illness with the exception of the terminal reticulocytosis and jaundice as due to a single condition, the patient's disease corresponds fairly well to nonleukemic myelosis or myeloid metaplasia, or to the leukoerythroblastic anemia described by Vaughan.

"Leukoerythroblastic anemia" is a term which might be applied descriptively to a number of different conditions. In the type of patient referred to by Vaughan there are osteosclerosis and a slight increase of fragility of the red blood cells. Dr. Thorson's patient did have a slight increase in the fragility of red blood cells but did not have osteosclerosis. Nevertheless, I have in mind another patient who five years ago, because of a large spleen and a slight increase in the fragility of red blood cells, called for a differential diagnosis between hemolytic jaundice and the condition that I think Dr. Thorson's patient had. Roentgen pictures of the bones of my patient were normal five years ago, but in roentgen films made in the past year areas of increased density have been shown. I suspect that fibrosis of the marrow and osteosclerosis are not necessarily present unless the disease has run a very chronic course. Is it possible that the condition in Dr. Thorson's patient represents a terminal exaggeration of the hemolytic process suggested by the observation of Vaughan that the red blood cells in such patients are abnormally fragile?

Although I believe that the proper diagnosis in this case is that suggested by Dr. Thorson, it is clear that there was terminally a hemolytic anemia which, as Dr. Dameshek has stated, can be properly described as acquired hemolytic jaundice. I should like to say a few words about why I believe that Dr. Thorson's patient had hemolytic jaundice as a terminal event. It is necessary to review briefly some recent work by Dr. Ham. He has shown that when normal human or animal blood is incubated sterilely in vitro, the erythrocytes progressively increase in volume, become more nearly spherical and exhibit increased fragility in hypotonic salt solution. Eventually, lysis of the erythrocytes occurs in the serum or in isotonic salt solution. The process can be accelerated by separating the red cells from the serum by centrifugation. If an imitation of this "erythrostasis" is produced in an animal by the injection of an agent which causes erythroagglutination and hence stagnation of red blood cells in certain organs, including the spleen, changes occur similar to those observed in the test tube. Thus, for example, the red blood cells in the spleen become progressively more fragile and even the circulating blood exhibits increase in fragility of erythrocytes in hypotonic salt solution. Hemoglobinemia ushers in acute hemolytic anemia. The agglutinating agent employed has no significant hemolytic properties in vitro. From these observations, supported by others which cannot be detailed here, Dr. Ham has come to the conclusion that hemolytic anemia may result whenever blood stagnates in the capillaries in the various organs in the body. In Dr. Thorson's patient difficulty was experienced in finding a suitable donor because of agglutination by the patient's serum. Autoagglutination of red cells has been reported in certain cases of Lederer's hemolytic anemia. The spleen of Dr. Thorson's patient showed extensive infarction. I would say, merely as a suggestion of the mechanism of the hemolytic anemia in this patient, that because of alteration of some components of the plasma there was increased agglutination of red cells and consequently erythrostasis in the spleen and in other organs. Experimentally, at least, Dr. Ham has demonstrated that agglutination of red blood cells in animals, induced by an agent not causing hemolysis in vitro, results in hemolytic anemia.

EDWARD A. GALL: I should like to lend a word of support for the clinicians. Some three years ago I had the privilege of viewing at autopsy a patient who five years before had a history practically similar to the one presented here except for the periodic thrombopenia. At that time a diagnosis of acquired hemolytic icterus was made and splenectomy performed. Without significant remission,

a slow but progressive decline ensued, and post mortem my associates and I found an extreme degree of hemosiderosis associated with marked erythropoietic hyperplasia of the marrow and to a less extent of the spleen, liver and lymph nodes. I think this case probably represented the same disease as the case presented by Dr. Thorson, and I simply want to establish the point that despite the reasonableness of the diagnosis of acquired hemolytic jaundice the urgency of splenectomy is not quite as evident as it is presumed to be.

S. B. THORSON: Every one has mentioned splenectomy, but I think opinion is well divided as to whether it should be done or not. Dr. Hickling reported a mortality rate of over 50 per cent, and in those patients who recovered there was no appreciable benefit.

WILLIAM DAMESHEK: Splenectomy has been done in a number of cases, roughly 30, of acute (idiopathic) hemolytic anemia of the acquired type. In about 23 of this group, recoveries were complete. For some reason the idea has spread that acquired hemolytic jaundice is not benefited by splenectomy. We concede that the condition is serious, but it is by no means hopeless. In our own series, 7 of 12 patients have made complete or incomplete recoveries.

A Primary Tumor of the Heart. HAROLD WOOD.

A 44 year old white woman presented cardiac signs and symptoms which suggested bacterial endocarditis. The roentgenologic study suggested disease of the mitral valve. She also had anemia, which conformed to the hypochromic normocytic type. Four weeks after the patient's discharge, left-sided hemiplegia developed, and she died in four days. Post mortem the heart was removed by the family physician and examined in the department of pathology at Tufts. In the left atrium, attached by a short broad pedicle to the margin of the obliterated foramen ovale, was a papillary tumor, 2.5 cm. in diameter, which overhung the mitral orifice. A detached papillary process of the tumor lay free in the left atrium. Much scarring of the left ventricular myocardium was evident. Tumor embolism of the brain was suggested as the cause of the hemiplegia.

Microscopically, the tumor consisted of a growth of endothelial-like cells, which originated from blood vessels of the endocardium and formed vascular-like structures. There were tumor emboli in the myocardium. The intercellular component of the tumor was an intricate meshwork of elastic and reticular fibrils, which were in close anatomic relation to the endothelial-like cells. The cells and fibrils were embedded in a mucinous ground substance. On the basis of the histologic picture and the site, the tumor was designated as primary endothelioma of the endocardium.

SIDNEY FARBER, *President*

BENJAMIN CASTLEMAN, *Secretary*

Regular Meeting, May 18, 1940

Myoepithelial Hamartoma of the Gastrointestinal Tract: A Report of Eight Cases with Comment Concerning Genesis and Nomenclature. B. EARL CLARK.

This paper was published in the Wolbach number of the ARCHIVES OF PATHOLOGY (**30**:143, 1940).

Multiple Carcinoma of the Trunk in a Case of Psoriasis. MORTIMER WARREN.

Tumors diagnosed as carcinoma were excised from the skin of a 47 year old man who had had psoriasis for thirty-five years and polycythemia for eight years.

The lesions were excised during a seven year period and were diagnosed as follows: 4 from the left lower quadrant of the abdomen, right groin and left scrotum, epidermoid carcinoma; 3 from the back, left groin and right thigh, basal cell carcinoma with areas of keratinization; 5 from the back, chest (2), thigh, elbow, basal cell carcinoma.

It was felt that the treatment of the psoriasis rather than the disease itself was probably the cause of the carcinomatosis. The patient had received treatment with roentgen rays and solution of potassium arsenite U. S. P. (Fowler's solution). Arsenic crystals could not be demonstrated in the tissues by the Osborne method.

DISCUSSION

LEON BALALIAN (by invitation): Carcinomatosis in association with psoriasis of the skin is unusual. In what proportion of the cases arsenic is responsible is still a question. Some authors believe that because there is no relation between the amount taken and the date of appearance of cancer other factors play a role. Some argue that psoriasis can develop into cancer spontaneously without any changes. Such an occurrence is very exceptional and a report of a case should be taken with a grain of salt. I wonder if the additional factor could not be one of the psoriatic treatments, such as vitamin or roentgen therapy. In America from 1 to 3 cases are reported each year, while in France there has not been a case reported for fifteen years. Psoriasis in France is treated almost wholly with a solution of potassium arsenite (Fowler's solution), whereas here roentgen treatments are frequently given in addition. Perhaps this fact explains the difference in the incidence of the cancer complication in these two countries.

SHIELDS WARREN: The interesting thing about this case to me is the indiscriminate way in which the epidermoid and basal cell cancers occur. Two lesions that it is almost impossible to distinguish clinically are epidermoid and basal cell carcinoma, regardless of what the etiologic factors may be. At the Collis P. Huntington and Palmer Memorial Hospitals in Boston and the Pondville Memorial Hospital in Wrentham, Mass., we have had to give up attempting to diagnose them clinically. We wait until we see what they are histologically. That is the important point, because of the possibility of the epidermoid carcinoma metastasizing and the great unlikelihood of the basal cell carcinoma doing so. I am inclined to agree with Dr. Warren that the condition in question is probably not related to the psoriasis.

Hemangioendothelioma of the Tibia with Metastasis to the Popliteal Artery. ROBERT FIENBERG.

Among authentic cases of hemangioma of bone, those in which there is involvement of the vertebrae and flat bones are fairly frequent, but those in which the long tubular bones are affected, especially by the malignant type of growth, are quite rare. I report a case. A 50 year old man had an authentic malignant hemangioma or hemangioendothelioma of the left tibia. The tumor was situated in the upper epiphysial region and invaded the joint cartilage. The neoplasm had destroyed the cortex, infiltrated the periosteum and metastasized to the popliteal artery at the level of the upper tibial condyle. There was no direct extension from the tibial tumor to the artery. An amputation in the lower midthigh region was recommended and done on the basis of the marked anaplasia, the pleomorphism of cells and the mitotic figures in the biopsy specimens. The main feature of the tumor was the blood vessels in varying stages of maturity; even in the most anaplastic regions traces of blood vessel formation were visible. The roentgen film of the tumor showed typical loculations, such as are seen in benign hemangioma; but in addition destruction of the cortex and invasion of the joint cartilage were visible. No other case like this could be found recorded in the literature. A plea is made to base the method of treatment on the anatomic site and the histologic details of the tumor in cases of hemangioma of bone.

DISCUSSION

SHIELDS WARREN: The problem of the proper therapy was most interesting. Several questions were raised; first, as to how sensitive the tumor would be; second, as to the likelihood of extension beyond the probable field of irradiation, and, third, whether there were enough cases of known malignant tumor in an extremity being cured by radiation to warrant passing up an attempt at amputation while the tumor was still localized. I think these things should be weighed in the consideration of any tumor of an extremity whether it involves the bony or the extraosseous structures.

A New Method for the Determination of Alcohol in the Breath Using Magnesium Perchlorate. W. W. JETTER.

A new apparatus for the determination of alcohol in the breath is described. Specifically, it finds the alcohol–carbon dioxide ratio in a sample of expired air. Magnesium perchlorate and ascarite are used for the absorption of the alcohol vapors and carbon dioxide, respectively. In 100 cases a good correlation between the blood alcohol concentration and the breath alcohol–carbon dioxide ratio has been observed. The advantages of this apparatus lie in its small size and compactness (weighing but 4 ounces [113 Gm.]) and the fact that it can be operated "on the spot" by the nontechnical person with but minimal training. Details will be published elsewhere.

BUFFALO PATHOLOGICAL SOCIETY

ERNEST WITEBSKY, *President*

SAMUEL SANES, *Secretary*

April 27, 1940

Agglutination Titers for Brucella Abortus After Vaccine Skin Tests in Apparently Normal Persons. NORMAN W. ELTON.

In order to establish the fallacy of a diagnosis of undulant fever based on a positive skin test and subsequent agglutination of Brucella organisms to a titer as high as 1:1,280, 8 normal persons were tested with a vaccine prepared by Hauenstein and used for skin tests with excellent results for several years. Each person received endermally 0.1 cc. of a suspension containing 4,000,000,000 killed organisms per cubic centimeter. The following day six had induration, edema and erythema greater than 4 by 5 cm. Two had negative results. All but one reaction had disappeared by the seventh day, and this one underwent necrosis and pitting, still evident two months later.

One week after the test agglutinins were not demonstrable in 2 subjects; the others were not tested at this time. Three weeks after the skin test agglutinins were microscopically demonstrable to a titer of 1:1,280 in all 8 subjects in one laboratory. In another laboratory, where only macroscopic slide agglutinations were read, the titers ranged from 1:80 to 1:320. In a third laboratory agglutination tests with killed Brucella abortus, living B. abortus and Brucella melitensis were carried out on all subjects. Six showed 4 plus agglutination in dilutions to 1:320 with one or all antigens. The person with the severest skin reaction yielded an agglutination titer of only 1:80, and the one with the weakest skin reaction, a titer of 1:160. Several prezone reactions occurred with the various antigens used by the third laboratory. The person who had no skin reaction had 4 plus agglutination in a dilution of 1:320. Another, whose skin reaction was so faint that it was interpreted as negative, gave a 3 plus agglutination of a living suspension of B. abortus in a dilution of 1:160.

Five weeks after the skin tests the agglutination titers were again determined by the first laboratory. Although the titers were less, the serum of 3 subjects still agglutinated to 1:1,280, and the serum of the remainder to 1:320.

The objective of this investigation was definitely attained in demonstrating that the agglutination test is not diagnostic after a skin test has been performed, even if the skin test was negative. The endermal vaccine test appears to be effective as a vaccination. I agree with those who interpret the skin test for undulant fever on the same basis as the tuberculin test, holding that it indicates merely previous contact with Brucella organisms and not active infection. All of these 8 persons have probably drunk raw milk at some time or other and can recall an occasional vague illness, but nothing recognizable as undulant fever. It is believed that these results should be considered also in testing cattle and that the sequence of skin test prior to agglutination test be avoided if a bacterial suspension is used.

Mode of Action of Sulfanilamide on Enterococci. ERWIN NETER.

It has been shown that sulfanilamide in a concentration of 1 per cent may inhibit the growth in broth of hemolytic and nonhemolytic enterococci and cause rather rapid loss of their viability. In an effort to elucidate the problem as to whether this sterilizing effect of sulfanilamide is due to a true bactericidal action of the drug or is the result merely of bacteriostasis, the following experiments were carried out.

Enterococci were cultured in infusion broth for eighteen to twenty-four hours at 37 C. The broth culture was centrifuged, and the sediment washed and resuspended in physiologic solution of sodium chloride containing phenol red as an indicator. This suspension of organisms was used for inoculation of both saline solution and saline solution containing 1 per cent of sulfanilamide. The mixtures were incubated at 43 C. Subcultures were made at frequent intervals. Two standard loopfuls of the mixtures were used for inoculation of the surface of blood agar plates, and the cultures were incubated at 37 C. for several days. In addition, in some of the experiments the number of viable enterococci was determined by means of poured blood agar plates.

The experiments revealed that enterococci when suspended in physiologic solution of sodium chloride and incubated at 43 C. lost their viability rather rapidly; with an inoculum of 2 loopfuls, viable organisms could no longer be demonstrated after a lapse of two to six and occasionally twenty-four hours. Even when a larger amount (0.5 cc.) of the original suspension which was incubated at 43 C. for twenty-four hours or longer was used for inoculation of broth, subcultures often remained sterile. The experiments, furthermore, showed that enterococci suspended in 1 per cent sulfanilamide dissolved in physiologic solution of sodium chloride lost their viability at approximately the same time as did the controls (three experiments); sometimes, viable organisms were demonstrable for a few hours longer in the sulfanilamide solution than in the saline solution (ten experiments); on the other hand, in six experiments the organisms survived somewhat longer in saline than in sulfanilamide solution.

From these experiments it may be concluded that sulfanilamide in a concentration of 1 per cent failed to reveal any striking direct bactericidal action on enterococci suspended in a nongrowth-promoting medium for three to twenty-four hours at a temperature of 43 C. This may be due to a number of different factors: 1. Sulfanilamide is bacteriostatic but not directly bactericidal. 2. The lag in the action of sulfanilamide may be longer than the time during which the enterococci remain viable in saline solution under the experimental conditions described. 3. Sulfanilamide may undergo some changes in the presence of organisms in a state of growth or of enzymatic activity that is not taking place when the organisms are suspended in physiologic solution of sodium chloride. 4. It is conceivable, and has been considered by several authors (Marshall, Wolff and Julius, and others), that sulfanilamide acts only on actively growing organisms.

In this, sulfanilamide contrasts with ordinary antiseptics, such as phenol. The experiments recorded here seem to point to the fact that some as yet unknown biologic activity of the micro-organisms is necessary if sulfanilamide is to be directly bacteriostatic and indirectly bactericidal.

Free and Combined Sulfanilamide in Human Bile. ROGER S. HUBBARD and RICHMOND K. ANDERSON.

The concentrations of free and of combined (presumably acetyl) sulfanilamide have been determined in bile collected from human subjects through T tube cannulas inserted after operations on the bile ducts. The method consisted in clarifying the bile with a mixture of trichloroacetic and phosphotungstic acids and determining the drug by the diazo method of Marshall and his co-workers, in a solution containing acetone. In the determination of combined sulfanilamide the filtrate was treated with hydrochloric acid and hydrolyzed for one hour in a boiling water bath before the diazo reaction was carried out. Both free and acetyl sulfanilamide (a synthetic product supplied by the National Aniline and Chemical Company was used) were added to bile and recovered quantitatively by this procedure, but the clarification could not be considered as wholly satisfactory, for a trace of bile pigment was present after precipitation in specimens from a fair number of the patients studied. The results, however, were concordant, and showed the following essential points. There was no compound in the clarified bile which gave a diazo reaction similar to that given by sulfanilamide. Shortly after 1 or 2 Gm. of sulfanilamide had been given by mouth, the drug appeared in the bile draining from the T tube, but never in concentrations equal to those of the sulfanilamide present in the blood. Sulfanilamide was present in the bile for twenty-four hours after the dose had been given. During the first four hours after the administration of such amounts, only the free form of the drug could be demonstrated in the bile. Thereafter both the free and the combined form were present, but the amounts of the latter were small, for only 10 to 20 per cent of the total sulfanilamide occurred as the combined derivative at periods when analyses of blood and urine showed that approximately half of the sulfanilamide in those fluids could be recovered only after acid hydrolysis. If acetyl sulfanilamide is formed in the liver, as current experimental work indicates, it is apparently not discharged in large amounts directly into the bile.

Injected Sucrose in Abnormal Extracellular Fluid. RICHMOND K. ANDERSON and ROGER S. HUBBARD.

Sucrose was injected intravenously and its concentration determined in ascitic, chest and edema fluids and in blood. Sterile fluids and blood serum were incubated with cultures of Bacillus coli communior and Bacillus coli communis and the sucrose determined by an appropriate modification of Roe's method for fructose. Since the former organism ferments sucrose while the latter does not, the difference in the two samples represents sucrose.

It was found that sucrose diffuses rapidly into these fluids and persists for relatively long periods. Normal persons had only traces present in the blood after twenty-four hours, while patients with abnormal accumulations of extracellular fluids had considerable amounts in their blood and in those fluids for a much longer time, some for over four days.

It therefore appears that abnormal extracellular fluids act as reservoirs for sucrose, so that it is retained in the tissues and at a relatively high level in the blood for long periods.

Polyposis of Appendix. S. SANES, D. F. PATCHIN and N. J. EICK.

A 20 year old white man complained of abdominal pain, attacks of vomiting and melena for three years. A freely movable and tender mass, which had shifted position at different examinations, was felt originally to the left of the umbilicus.

In a series of gastrointestinal roentgenograms the appendix was not visualized; there were pain and tenderness in the right lower quadrant of the abdomen. At laparotomy a pedunculated adenomatous polyp, which caused obstruction of the jejunum, was excised. The appendix was removed because it appeared enlarged and injected. Examination revealed two large adenomatous polyps, 3.7 cm. and 1.7 cm. long, with cystic dilatation of glands, fecal concretions in glands and inflammation of stroma. Otherwise the mucosa showed glandular hyperplasia and adenomatosis.

NEW YORK PATHOLOGICAL SOCIETY

MAURICE N. RICHTER, *President*

D. MURRAY ANGEVINE, *Secretary*

Regular Monthly Meeting, May 23, 1940

A Case of Extragenital Choriocarcinoma in the Male. HOUSTON W. SHAW (by invitation).

Two theories of the origin of extragenital choriocarcinoma have been advanced: (1) It develops from germinal rests; (2) it is due to malignant transformation of teratoma. Staemmler (*Verhandl. d. deutsch. path. Gesellsch.* **27**:190, 1934) supported the first theory by finding testicular rests at the root of the mesentery in 2 of 13 routine autopsies. A review of the literature reveals only 4 cases in which a neoplasm was proved to be extragenital choriocarcinoma; a fifth is now added.

A physician, 45 years of age, entered New York Post-Graduate Medical School and Hospital, Aug. 7, 1939, with severe pain in the left lumbar region. The illness had begun in June with dull pain in the left flank, polyuria and pain in the right breast. A urogram revealed a rotated left kidney, slight obstruction of the ureter and a suggestion of a medial mass. Both red and white blood cells were noted in the urine. In July a mass was palpable in the left flank at the lower pole of the kidney, in view of which exploratory operation was advised.

This man stated that he had had left renal colic with passage of calculi in 1932. The left breast was removed in December 1938; the diagnosis was fibroadenoma.

The findings of note were a temperature of 101 F., the mass in the left flank and diffuse enlargement of the left breast. At operation, August 7, a retroperitoneal tumor was found near the lower pole of the left kidney. Coma intervened with bronchopneumonia and death occurred August 19. The specimen removed at operation was diagnosed as choriocarcinoma. The next day the Aschheim-Zondek test was negative.

Autopsy.—In the left retroperitoneal region was a large hemorrhagic neoplasm, composed of red and gray-brown hemorrhagic tissues with thick communicating septums and blood spaces. Enlarged lymph nodes in the region had fused with the neoplasm and compressed the aorta and the renal artery. Posterior and lateral to the kidney was a large abscess which communicated with the tumor and with the lumbar operative wound. The lower pole of the left kidney was compressed by the tumor, and several large infarcts were noted. The lungs contained many metastatic nodules, which were similar to the primary tumor. The right breast was enlarged, and there were numerous, densely packed shiny pinhead-sized gray areas in an area 4 by 2 by 1.5 cm.

The retroperitoneal tumor was composed of strands of fibrous tissue, in part necrotic. There were large numbers of tumor cells resembling those of chorionic villi, i. e., large polyhedral cells with vacuolated cytoplasm and large vesicular nuclei (Langhans' cells) and large syncytial masses with multiple

hyperchromatic nuclei. The nodules in the lung were of the same structure. In the breast connective tissue proliferation was marked, with nests of glandular tissue lined by multilayered cuboidal epithelium and surrounded by lymphoid and plasma cells. The testes were cut into blocks 2 to 3 cm. thick, and the section from each block showed no tumor or scarring. There was atrophy of many tubules, and in areas the interstitial cells were prominent. Because no tumor could be found in the testes, the growth is considered to have arisen from germinal rests in the retroperitoneal region.

DISCUSSION

JACOB HEIMAN: Were there any gross or microscopic changes in the adrenals or the pituitary?

MAURICE N. RICHTER: If one wants to eliminate all possibility of a primary tumor in the testes, it is necessary to make an adequate examination in the fashion described.

PAUL KLEMPERER: Some years ago my colleagues and I studied a case of mediastinal teratoma, and we followed exactly the procedure outlined, because Prym claimed all cases of extragenital choriocarcinoma reported had to be considered as doubtful inasmuch as the testes were not properly examined. I think it is absolutely necessary in cases of extragenital choriocarcinoma to examine the testes very carefully for tumor. Two weeks ago I saw a case of testicular teratoma with most extensive metastases throughout the organs, also choriocarcinomatous in nature, and the tumor in the testis was no larger than a bean. One can understand that if such a tumor should become necrotic and heal, leaving only the metastases, the condition would be considered extragenital choriocarcinoma, although this would be erroneous.

HOUSTON W. SHAW: The adrenals were normal both grossly and microscopically. Unfortunately, the pituitary could not be examined.

A Case of Massive Urolithiasis Following Massive Doses of Heparin and Sulfapyridine (2-[Paraaminobenzenesulfonamido]-Pyridine) for Subacute Endocarditis Due to Infection with Streptococcus Viridans. ARTHUR SCHIFRIN and (by invitation) SAUL KELSON.

A 21 year old woman had had rheumatic valvular disease since childhood, with good cardiac compensation. She was admitted to the hospital after a febrile illness of three months, during which chills, arthralgia, Osler's nodes, and pains in the finger tips had been noted. Two blood cultures had shown Streptococcus viridans, 20 colonies per cubic centimeter. Classic signs of subacute bacterial endocarditis were present. She was observed for sixty-three days and received a total of 261 Gm. of sulfapyridine. Renal pain on the left side, gross hematuria and dysuria were present from the fourth to the ninth day and began after only 24 Gm. had been given. In the second period (forty-eighth to sixty-third day) she received 122 Gm. of the drug. The sodium salt was given as a continuous drip. Mild congestive failure occurred in the first period and was quickly relieved by digitalization. In the last four days there was severe congestive failure, which was unrelieved by digitalization, and was the cause of death. Uremia was not present; the urinary output varied from 33 to 44 ounces (about 1½ to 1 liter). Sulfapyridine had no influence on the bacteremia or febrile course. Vomiting was not an outstanding feature except terminally. A continuous intravenous drip of heparin had been given from the first to the fifth day, from the sixteenth to the twenty-third day and throughout all the last fifteen days. No hemorrhages attributable to heparin were noted. Post mortem there were found subacute bacterial endocarditis, with vegetations on the aortic and mitral valves, old rheumatic mitral valvular disease, chronic passive congestion and a tumor and infarcts of the spleen. Each kidney weighed 150 Gm.; the capsules stripped easily; the surfaces were smooth, and the vessels were markedly congested. The pelves, though only slightly dilated, were completely filled by innumerable small

calculi held together loosely by fine spider web-like fibrinous material. The calculi themselves varied from pinhead to split pea size. Many formed sievelike and conglomerate mushy putty-like friable masses. Their color varied from gray to pink or brown. Some calices showed only slight dilatation. The papillae were not hemorrhagic. Broad isolated and confluent submucosal hemorrhages in the pelves and calices were seen. The ureters showed slight fusiform, irregular dilatation and contained a few calculi and scattered debris. The ureteral walls were slightly edematous and ecchymotic. The bladder wall showed scattered petechiae. Fine calcareous material and gravel floated about in the pink urine of the distended bladder. Wet smear crushings of the calculi showed numerous colorless, pink and brown crystals of two types: (1) wedge shaped with the base and one side straight, the third being ragged with needle-like projections; (2) flatter and more polygonal. Chemical assay (by Dr. J. V. Scudi) of washed calculi showed 82 per cent acetylated, and 3 per cent free, sulfapyridine.

Microscopic examination showed: no renal infarcts; very rare embolic glomerular lesions; marked interstitial congestion; slight degeneration of tubular epithelium; no significant dilatation of tubules or glomerular spaces; submucosal hemorrhages in the pelves, calices and ureters, with fine areas of cell infiltration. One arteriole in a calix showed necrosis.

The experimental observation of Antopol and Robinson that urolith formation may occur without initial degeneration or vascular changes within the renal parenchyma is confirmed for the human subject. Acetylation of sulfapyridine may occur both rapidly and to a high degree in man. Excretion-thrust factors can influence the massiveness of the urolithiasis, and this indicates the necessity of cautious control of the dose and rate of administration of the sodium salt. Irrevocable cardiac failure may be induced by the large volumes of intravenous fluids hitherto used in the administration of heparin or of the sodium salt to patients with cardiac disease. Even with doses of sulfapyridine and heparin which are at present considered to be full and adequate, failure may occur in controlling the bacteremia, the febrile course or the local lesion of subacute bacterial endocarditis caused by Streptococcus viridans.

DISCUSSION

LAWRENCE H. COTTER: What were the levels of sulfapyridine in the blood?

SAUL KELSON: This is the only sulfapyridine urolithiasis encountered in the 24 patients with subacute bacterial endocarditis whom I have treated so far with sulfapyridine and heparin. In several patients sulfapyridine concentrations of 15 mg. per hundred cubic centimeters of blood or higher have been maintained.

The commonest event which has raised the question of sulfapyridine stones is renal infarction, with pain in the flank and hematuria. One patient receiving the combined treatment, a woman of 64, showed severe gross hematuria shortly after a mercurin-theophylline preparation (mercupurin) had been given to relieve congestive heart failure. Sulfapyridine lithiasis was strongly suspected, but if the use of that drug had to be discontinued, the woman would lose her only chance for recovery. It seemed possible that the mercurin-theophylline, which may cause microscopic hematuria in a patient not receiving heparin, might, with the clotting of the blood greatly decreased by heparin, have set off gross hematuria. We were in a dilemma, but the second assumption seemed more hopeful, if less likely; the use of heparin was discontinued briefly, the hematuria cleared, and it was concluded that sulfapyridine stones were not present.

I show a lantern slide of a patient with typical subacute bacterial endocarditis, who, following therapy, remained free of the disease for six months. He did suffer, however, from rheumatic fever, which aggravated the advanced cardiac damage, and at the end of the six months he died in congestive heart failure. On the aortic flap of the mitral valve may be seen a large lesion with the characteristic configurations of subacute bacterial endocarditis. It is completely healed, however, and calcified, and careful search by crushings and cultures have failed to show any organisms.

JESSE G. M. BULLOWA: Massive doses of sulfapyridine are not necessary for lithiasis to occur. A case was called to my attention in which the administration of 20 Gm. of sodium sulfapyridine was followed by lithiasis, hematuria and azotemia.

REUBEN OTTENBERG: Last August I saw a death from urinary suppression in a man of 29 who was treated for pneumonia with a total of only 14 Gm. of sulfapyridine in two days. Hematuria occurred first, and then, a day later, total suppression, and the patient died with uremia. Post mortem there was a black grumous mass of hemorrhage and crystals in the renal pelvis.

Experimental Aspects of Sulfapyridine Urolithiasis. WILLIAM ANTOPOL.

Changes produced by sulfapyridine in the urinary tracts of rats, rabbits and monkeys (Antopol, W., and Robinson, H.: *Proc. Soc. Exper. Biol. & Med.* **40**: 428, 1939) are reviewed. These changes are interpreted as due to the insoluble acetyl compound, which is precipitated out in the kidneys. It is pointed out that in mice and dogs, species in which acetylation does not occur appreciably, concrements cannot be produced. If, however, acetylated sulfapyridine (as a sodium salt) is injected intravenously into mice, precipitation occurs in the pyramids of the kidneys. The same localization is found in rats.

The formation of glycuronic acid compounds, as described by J. V. Scudi (*Science* **91**:486, 1940), is mentioned as a mechanism counteracting the formation of stones by introducing an element of competition between this highly soluble derivative and the poorly soluble acetylated sulfapyridine. As both these compounds are formed in the liver, conditions in this organ may play an important role in the formation of renal calculi.

Severe renal damage produced in rats by intravenous injection of 0.85 Gm. per kilogram of sodium acetylated sulfapyridine was reflected in the urinary findings. An initial stage of oliguria with retention of urea in the blood was followed by most pronounced polyuria and hyposthenuria. Within a short period (twenty-four hours) the urine became loaded with red blood cells and contained considerable amounts of albumin. These changes gradually subsided in the course of a few days. During that period only small amounts of the acetylated drug were eliminated from the body (precipitation in the kidneys).

Sodium sulfapyridine in equimolecular amounts (0.75 mg. per kilogram) did not produce these severe urinary changes except for the appearance of moderate numbers of red blood cells. In contrast to the acetylated compound, free sulfapyridine was rapidly eliminated.

Twenty-four hours after the administration of acetylated sulfapyridine the papillary ducts of the kidneys were blocked by massive precipitation of the drug, while the cortex showed punctate hemorrhages due to necrotizing nephrosis. In forty-eight hours the renal cortex appeared studded with white dots as a consequence of extensive calcification of the distal convoluted tubules and the collecting tubules. These lesions remained visible even many days after the injection, whereas no uroliths or renal damage save congestion was found after a single injection of free sodium sulfapyridine.

DISCUSSION

S. M. RABSON: In those animals that survived the longest period was there any evidence of change in the pelvic epithelium?

J. M. RAVID: Recently I reported here the case of a 79 year old man who died as a result of sulfapyridine therapy, after a total dose of only 8 Gm. of the drug had been consumed. The lesions were mainly renal and consisted in a plugging of the tubules with hemoglobin pigment and casts and a moderate degree of degeneration of the tubular epithelium. This process was secondary to acute hemolysis with its concomitant jaundice. It is interesting that in one of the sections I found a few casts each with a shell of deep-staining material around it which had the appearance of calcium.

Regarding the experiments by Dr. Antopol, I noticed that very little of the tubular change seen in my case was observed by him. I should like to ask whether he found any evidence of such change and also whether he found any hemosiderin pigmentation of the renal tubules and of the reticuloendothelial elements of the liver and spleen. I should like also to ask whether he can explain why this drug has produced in his animals, as well as in Dr. Schifrin's patient, such marked urinary lithiasis, while in other patients, including my own, its main effect was hemolytic. I noticed in his experimental series that practically every animal had hematuria. I wonder whether this is not indicative also of hemolysis comparable to the process which has been observed in my case as well as in others.

MAURICE N. RICHTER: It would be interesting to know how accurately the crystals in the tubules can be identified by histologic methods. Crystalline deposits, concretions of various types, are not at all uncommon. Some of the concretions in the pictures shown are not unlike those described by Dr. Hans Smetana, for example, in carbon tetrachloride poisoning, or those seen in various renal changes after metallic poisoning. Are the crystals anisotropic? Again, is any special technic required for their demonstration in tissues? It has been pointed out that these crystals are found only after the use of a very rapid technic of preparing tissues with celloidin (a pyroxylin preparation) and are not found with the usual celloidin technic, indicating that the solubility of the crystals prevents their demonstration in ordinary preparations. Again, how long do the crystals remain in the kidney? How long can they be identified after the sulfapyridine therapy has been stopped? Some of these cases may be of medicolegal interest, especially when sulfapyridine has been given in the presence of renal disease.

WILLIAM ANTOPOL: In reply to Dr. Rabson, there were no areas of metaplasia in the renal epithelium. There were atrophic areas and areas which contained a number of mitotic figures. In answer to Dr. Ravid, little hemoglobin pigment was present in the sulfapyridine-treated animals. There has been, however, a series of experiments on chronic intoxication with sulfanilamide in which the renal and splenic lesions originally described may answer the question (Antopol, W.: *J. Pharmacol. & Exper. Therap.* **65**:412, 1939). It was further found that after long administration of sulfanilamide the resistance of the red blood cells becomes markedly increased and the spleen tremendously enlarged and bulky. Microscopic examination of the spleen shows typical hyperemia, as seen in hemolytic jaundice. It is believed that sulfapyridine might in the same way alter the membranes of the red cells, which are then engulfed by the spleen, despite the increased resistance of the cells. I do not think that this phenomenon is related to urolithiasis except that the precipitated hemoglobin may in turn initiate precipitation of crystals. Why one animal or patient should have urolithiasis and another hemolysis one cannot say. So far as urolithiasis is concerned, there is considerable individual variation in the amount of acetylation. Dr. Scudi's finding is, however, very important, and if a method can be devised by which most of the sulfapyridine can be converted into a glycuronate, the problem will be solved. I can only agree with Dr. Schifrin that it is the thrust of the acetyl sulfapyridine excretion which is most important in determining whether urolithiasis will be present or not.

One knows that substances in the urine become concentrated as they pass through the tubules of the kidney. In addition, the reabsorption of dextrose in the tubules (acetyl sulfapyridine is considerably more soluble in a dextrose solution) and the change from p_H 7.2 to 4.5 in the collecting tubule might be elements which exaggerate the precipitation due to the important factor of concentration. Since it appears that acetyl sulfapyridine is cleared through the glomerulus much faster than sulfapyridine, and the belief is that sulfapyridine and not acetyl sulfapyridine might be reabsorbed in the tubules, the predominance of the acetyl derivative in the uroliths is understandable. I believe that hemolysis plays no important role in the hematuria, since intact red blood cells are found in the urine.

Concerning the identification of the concretions in the urine of patients which Dr. Richter asked about, the crystals take on many bizarre forms. I cannot answer the question about polarized light; I have had no experience with it. Concerning the rapid celloidin technic, Dr. Stryker describes basophilic round masses which might be calcific or protein in nature.

How long after the administration of sulfapyridine is stopped can one detect the crystals in the urine? That will depend almost entirely on the rate of the washing out of the sulfapyridine from the tubules of the kidney and the urinary tract. In animals receiving massive doses of acetylated sulfapyridine it is still present in the collecting tubules of the kidney after four days despite marked diuresis. In other animals receiving sulfapyridine and not the acetylated form we found numerous red blood cells, and yet four days after a single dose (and all of these animals received but one dose) there was no evidence of precipitation or stone formation in the kidney, pelvis or ureters. I believe that in most patients, even with massive doses, most of the drug should be washed out within a period of three, and at the most four, days.

Color Photography at the Autopsy Table, with Illustrations. JACOB WERNE, HANS FREIVOGEL (by invitation) and CHARLES BREEDIS (by invitation).

The substance of the technical part of the presentation has been published elsewhere (Freivogel, H., and Breedis, C.: *J. Biol. Photographic A.* **8**:173, 1940). The photographic equipment consists of a Leitz rotating focusing attachment which permits photographing the whole or parts of a body and the viscera in situ. Two Leica cameras are used interchangeably, one loaded with kodachrome "A", the other with a panchromatic fine grain film. The polarizing device consists of two Eastman Kodak polascreens mounted between glass in two wooden frames, to which are attached, by brass clasps, aluminum reflectors containing photoflood no. 2 bulbs. Another rotatable Eastman Kodak polascreen is placed in front of the camera lens. By turning this screen and viewing the image in the ground glass, one can control the amount of high lights.

DISCUSSION

MAURICE N. RICHTER: Dr. Otani has some colored photographs taken by another method. It would be interesting to see them for comparison.

There is no question about the advantage of color photography for certain preparations, as there are some things which cannot be shown well in black and white. I have been using Kodachrome film, as I do not have to develop it. For certain types of work black and white has advantages. If one wants to put paper prints in the records, it is easier with black and white.

I should like to ask whether Dr. Werne knows anything about the keeping qualities of Kodachrome. We have been taking Kodachrome photographs in the hope that in five or ten years they will be as good as they are now and will not have lost their color. From the experience of others I am not sure that this will be the case.

(Dr. Sadao Otani showed Kodachrome photographs taken with a Kine-Exakta camera without using polarized light, through a diffusing screen made of celluloid material on a ground glass surface, using two photoflood bulbs as the source of illumination. The method is an extremely simple one.)

JACOB WERNE: Our technic makes provision for black and white photography. By simply changing from one Leica camera to another we accomplish this result without additional focusing. An integral part of our records is the corresponding collection of black and white pictures.

I am impressed with the excellence of Dr. Otani's pictures but feel that accuracy of reproduction requires control of the amount of high lights, and this control can be maintained by our technic.

I also emphasize the ease with which we can take routine pictures at the autopsy table. The mechanics of our method permits rapid changing and cleansing of the fields. The relative inexpensiveness of this technic permits its more extensive use and hence its wider application for conference, teaching and medicolegal purposes.

HANS FREIVOGEL (by invitation): As to how long these Kodachrome pictures will last, I can say only what the Eastman Kodak Company has told me: The colors should last indefinitely if the slides are kept in a cool, dark, dry place. Sunshine discolors them.

With regard to Dr. Otani's technic, I must compliment him on the simplicity of his setup and the excellent pictures he has obtained with its use; however, I feel that it does not permit the latitude of use that our setup does because, according to our technic, we can take pictures in situ and of all or parts of the body, using polarized light, as easily as of the eviscerated specimen. The cleansing of the field also is important if one is to get accurate and pleasing results, and it seems to me that Dr. Otani's enclosure limits the extent to which these operations may be carried out.

LESTER KAHN (by invitation): I address you as a commercial photographer whose chief concern is making photographs for advertising. Kodachrome film is one of the outstanding technical achievements of recent years and has earned a definite place in the advertising field. In such work it is obvious that pleasing effects are of prime importance, whereas in your work fidelity to fact is paramount.

In the preparation of pictures for advertising such articles as cosmetics or soaps, for example, we sometimes encounter the need for pictures analogous to those required in medical illustration. The slides that I have seen here have fascinated me, both because of their technical excellence and because of the ingenious methods used to obtain them.

Polarized light, a new and not well known medium, has received insufficient attention. It offers great advantages in eliminating the obliterating glare from wet surfaces and simplifies the rendition of textures coated with mucus or other light-reflecting fluids.

The best way to control this is by the use of polarized light sources in conjunction with polarizing devices fitted to the lens. Although this is practical for postmortem specimens, the reduction in light intensity may require too long an exposure for work on patients. It is possible to improve textural rendition with a polarizing screen on the lens without seriously increasing the time of exposure. I strongly recommend that you carefully examine the effects obtainable with the lens polarizer alone before going to the expense of complete polarization.

The effect of lens polarization can be studied by holding the polarizing screen in front of the eye and slowly rotating it. When a satisfactory rendition appears, the greatest care should be taken to see that the screen is placed on the lens in the correct position. The best way to use a polarizing screen on the lens is by means of a camera having a ground glass where this effect may be accurately adjusted. I have seen renditions of teeth in which the complete elimination of surface high lights imparted a plaster-like quality to the enamel, which falsified the natural appearance. The essential consideration is the purpose to be served. Then utilize one or the other or both of the technics shown.

I suggest that you bring some of your photographic problems to the better known among commercial photographers. They have been forced by the exigencies of their work to achieve a high degree of craftsmanship, and I am certain that most of them will be glad to help you with your problems.

I should like to mention a camera recently developed by Dr. Gustav Bucky which is completely self contained and perfectly adapted to the most difficult color photography of the human body. This instrument sets its own exposure, adjusts its own diaphragm, automatically focuses itself and carries its own light source.

Book Reviews

Neoplastic Diseases. By James Ewing, A.M., M.D., Sc.D., LL.D., professor of oncology at Cornell University Medical College; consulting pathologist at Memorial Hospital. Fourth edition, revised. Cloth. Pp. 1160, with 581 illustrations. Price $14. Philadelphia and London: W. B. Saunders Company, 1940.

The long-awaited new edition of Ewing's "Neoplastic Diseases," "revised and enlarged," is here at last. The first edition appeared in 1919, the second in 1922 and the third in 1928. The present edition has 33 more pages and 35 more illustrations than the third. Many sections have been extensively revised, some chapters completely rewritten and others shortened, but the general plan and scope of the book remain unchanged.

There are two parts, "General Oncology," 155 pages, and "Special Oncology," 917 pages. The topics in the first part are: history of cancer; definition, classification, general pathology; malignancy of cancer, its effect on the organism; metastasis; chemistry of tumors, serology; theories of the nature of cancer; the special etiology of tumors, trauma; the parasitic theory; experimental cancer research (27 pages). The chapter on experimental cancer research has been enlarged to include a brief review of cancerigenic hydrocarbons and of chicken sarcoma. The comment on the work with cancerigenic hydrocarbons is of interest: "It is obvious that a new and fundamental approach to the pathogenesis of some cancers . . . has been opened up by these investigations. The exact scope of its application remains for the future to determine." It is inconceivable that the great efforts in experimental cancer research will result in accretions only to the general knowledge of animal cancer without any useful application to the understanding, prevention, diagnosis and treatment of human cancer.

The second part, "Special Oncology," deals with the varieties of tumors and the tumors of the particular organs, and the last chapter with teratologic oncology (teratoma, dermoids, mixed tumors). This part is in fact a series of highly instructive, well rounded, readable reviews of the different neoplastic diseases in which the fundamental nature and course of tumors are correlated with their clinical manifestations in order to pave the way as far as possible at this time toward the recognition of the characteristics of each neoplastic entity.

As in previous editions, the bibliography is placed at the end of the book. The references are given according to authors and text pages, the names of the periodicals being abbreviated greatly. There is an incomplete key to the abbreviations. The titles to books cited are abbreviated also in many cases. This valuable bibliography would have been more easily useful if the references had been placed either at the end of the chapter or at the bottom of the page, with standard abbreviations of the names of periodicals. As it stands, the bibliography covers well the literature on human tumors up to recent times. Only rarely are articles of authors referred to in the text not found listed in the corresponding part of the bibliography (e. g. Haga, p. 135; Weller, p. 872).

The following quotations illustrate clearly how changes in the views about the origin of tumors develop: Page 81: "While this [Cohnheim's theory] is not a theory of universal application, yet that embryonal cells possess more than any others the essential features of tumor growth is, perhaps, the most important single fact in our knowledge of tumor genesis." Page 84: "Thus it becomes evident that Cohnheim's theory, while it explains the structure and occurrence of many tumors, wholly fails to reveal why the embryonal cells begin to grow and when growing produce malignant tumors instead of normal structures." Page 126: "The results in this field [the parasitic] show that parasites may be concerned only with the inception of certain tumors, but that their influence

cannot explain the continuous autonomous growth of malignant neoplasm wherein lies the real mystery of the cancer process." Page 526: "Clinical observation has long indicated that the majority of tumors are not dependent on congenital abnormalities in tissue structure, but arise from once normal, but previously altered, tissues, and that various forms of chronic inflammation are observed to precede the appearance of most tumors."

It is perhaps inevitable that the assimilation of new knowledge into old chapters cannot always be smooth and balanced. And naturally the older literature at times may seem to be summarized better and more completely than the recent, steadily growing accumulations. It is notable, for instance, with respect to Fibiger's work, that the newer interpretations of its significance, to which incomplete reference is made on page 138, have not led to any qualification of the statement on page 123 that "Fibiger has fully demonstrated the relation of Spiroptera to a gastric carcinoma in rats." In connection with the statement (page 107) that "radium deposits in the bones lead to osteogenic sarcoma" one misses any mention here or in the section on osteogenic sarcoma of the detection of radioactivity in persons who have been exposed to radium. The discussion of gastroscopy in the diagnosis of gastric cancer is limited to the interesting article on the subject by Janeway in 1913; the newer developments of gastroscopy are not considered. On pages 1014 and 1015 are obvious mistakes in the figures purporting to give the size and weight of the hypophysis.

The book is inconsistent in its use of the word "epithelioma," which commonly has been applied to carcinoma developing from surfaces lined with squamous cells and by metaplasia of other epithelial cells. On page 524 it is stated that "epithelioma" "should doubtlessly be displaced in favor of the designation 'epidermoid carcinoma.'" In the two chapters on epidermoid carcinoma, as well as elsewhere, "epithelioma" occasionally creeps in; and in the chapter on chorioma, "choriocarcinoma" is used consistently in the text but "chorio-epithelioma" in the running heads.

The illustrations are almost exclusively photographic and mostly original. They are, with perhaps an occasional exception, highly instructive. The photomicrographs as a rule reproduce accurately the microscopic fields they represent.

This book, which is assured of a cordial welcome, continues to be a critically constructive summary of the knowledge of human neoplastic diseases and a superb guide to the application of that knowledge of facts and principles to their prevention, diagnosis and treatment. No single volume now available covers that important field so comprehensively and competently as Ewing's fourth edition.

The Electrocardiogram in Congenital Cardiac Disease. A Study of 109 Cases, 106 with Autopsy. Maurice A. Schnitker, B.Sc., M.D., Associate Attending Physician, Toledo Hospital; Junior Staff Physician, Lucas County Hospital; Member Active Staff, St. Vincent's Hospital, Toledo, Ohio. Cloth. Pp. 147, illustrated. Price $3. Cambridge, Mass.: Harvard University Press, 1940.

Down to comparatively recent times congenital malformations of the heart attracted attention chiefly as pathologic curiosities, though the revelations of the necropsy were sometimes suspected by the physician when, in the newborn, cyanosis and dyspnea were especially marked. With the advent of the stethoscope and physical diagnosis, the development of embryology, the more careful recording of postmortem observations, the aid from roentgen examinations, the more sharply defined classifications and more extended studies, such as those of Maude Abbott, congenital heart disease has acquired an interest that is not alone academic, but practical. For it is now known that diagnosis of the condition, though by no means always accurate, is not impossible, and that prognosis is not infrequently surprisingly favorable. Lately, also, encouraging results have been reported by surgeons who, like Robert E. Gross, have operated on patients with patent ductus Botalli. And there is a ray of hope that treatment by sulfanilamide

or some of its congeners, perhaps in conjunction with heparin, may be helpful in preventing or curing the subacute bacterial endocarditis that is prone to appear at the site of congenital defects. Therefore, one welcomes this volume which records an attempt to discover what forms of electrocardiogram are met with in the study of various types of cardiac malformation. After a search of the literature, Schnitker submits an analysis of 109 cases, with electrocardiograms, in 106 of which there have been autopsies. Naturally, the histories and necropsy protocols vary in quality, yet the author's abstracts are fairly satisfactory as are his interpretations of the 108 electrocardiograms, most of which are well portrayed. He frankly admits that the tracing is but one aid to diagnosis, which in the main must depend on the history, the physical examination and the use of the roentgen ray. He further admits that the frequency with which congenital lesions are combined often makes it difficult or impossible to reach a clearcut diagnosis. Specific electrocardiograms are almost unknown. Hard and fast rules for interpreting cannot be laid down. Yet, as he says, "the more refined and exact the diagnosis, the more accurately can we prognosticate and the more intelligent will be the management of the patient with the malformed heart." With certain lesions the evidences in the tracings of right axis deviation are suggestive. A tendency toward biphasic complexes has been often noted when septal defects have been present. Sharp P waves or exaggerated amplitudes in several leads have also been regarded as suggestive. Altogether, the conservative attitude of the author is to be commended. The work should inspire the clinician to greater efforts toward refined diagnosis. He may at times in cases of supposed obscure or atypical symptoms be led to suspect the existence of congenital defects. Pathologists will be influenced to describe more minutely the location, extent and character of these congenital cardiac lesions. It is to be hoped that the author and others will carry on these studies in the future, for they seem to be well worth while.

Gynecological and Obstetrical Pathology, with Clinical and Endocrine Relations. Emil Novak, A.B., M.D., D.Sc. (Hon. Dublin), F.A.C.S.; associate in gynecology, Johns Hopkins Medical School; gynecologist, Bon Secours and St. Agnes' Hospitals, Baltimore. Cloth. Pp. 496, with 427 illustrations. Price $7.50. Philadelphia and London: W. B. Saunders Company, 1940.

In the preface the author states that this book represents his "concept of the composite needs of the student, practitioner, specialist and pathologist" in the field of gynecology and obstetrics. He also points out that "all but a few very rare lesions of the female genital organs" are discussed. Malformations, except placental, are not even mentioned, and the pathologist will look in vain for any description of injuries caused in abortion. The first chapter deals briefly with reproductive endocrinology and serves as an introduction to later discussions of the cyclic changes in the female genital tract. Then follow sections on the vulva, the vagina, the uterus, the tubes, the ovaries, ectopic pregnancy, pelvic endometriosis, placentation, hydatidiform mole and choriocarcinoma, the final chapter (L. M. Hellman) describing the abnormalities and diseases of the placenta and appendages. At the end of each chapter is a short list of selected references. The illustrations are excellent, mostly original photographs of microscopic appearances. Special mention must be made of the value of the photographs showing the structure of various forms of cancer and of the ovarian tumors of special current interest. Reference is made to the histologic grading of carcinoma, epidermoid as well as glandular, and here there is room for more and better illustrations of the different grades, especially of adenocarcinoma. The author would have been quite consistent in his nomenclature for cancer had he used "choriocarcinoma" in place of "chorionepithelioma." Within the limits set, the book summarizes well and clearly the present knowledge in its field. Outstanding features are the descriptions of the cyclic histologic changes in the genital tract and of the neoplasms of the uterus and ovaries. The book meets well the needs set forth by the author in his preface.

Books Received

A Comparative Study of the Seasonal Incidence of Mortality in England and Wales and in the United States of America. E. Lewis-Faning. Medical Research Council, Special Report Series, no. 239. Paper. Pp. 69. Price 30 cents. New York: British Library of Information; London: His Majesty's Stationery Office, 1940.

Graduate Medical Education: Report of the Commission on Graduate Medical Education, 1940. Cloth. Pages 303. Chicago: University of Chicago Press, 1940.

Modern Dermatology and Syphilology. S. William Becker, M.D., associate professor of dermatology and syphilology, Kuppenheimer Foundation, University of Chicago, and Maximillian E. Obermeyer, M.D., assistant professor of dermatology and syphilology, Kuppenheimer Foundation, University of Chicago. Cloth. Pp. 871, with 493 illustrations. Price $12. Philadelphia and London: J. B. Lippincott Company, 1940.

Travaux cliniques. D. Zamfir, Hôpital "Regina Elizabeth" de Bucarest. Paper. Pp. 142. Price 80 cents. Paris: Masson & Cie, 1940.

La circulation de retour. A. Jouve and J. Vague. Préface de Laubry. Paper. Pp. 180. Price 70 cents. Paris: Masson & Cie, 1940.

Studies in Nutrition: An Inquiry into the Diet of Families in the Highlands and Islands of Scotland. E. P. Cathcart, A. M. T. Murray and J. B. Beveridge. Medical Research Council, Special Report Series, no. 242. Paper. Pp. 37. Price 25 cents. New York: British Library of Information; London: His Majesty's Stationery Office, 1940.

Dental Disease in the Island of Lewis. J. D. King. Medical Research Council Special Report Series, no. 241. Paper. Pp. 61. Price 30 cents. New York: British Library of Information; London: His Majesty's Stationery Office, 1940.

The Structure of Teeth as Shown by X-Ray Examination. J. Thewlis, of the physics department, National Physical Laboratory, Teddington, Middlesex, England. Medical Research Council, Special Report Series, no. 238. Paper. Pp. 81, with 51 illustrations. Price 75 cents. New York: British Library of Information; London: His Majesty's Stationery Office, 1940.